BACHELOR THESIS

BIOMEDICAL ENGINEERING

Pressure measurement using bubbles and ultrasounds

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The art and science of asking questions is the source of all knowledge.

- Thomas Berge

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Acronyms

ePHD - Extended pure harmonic detection

HTP – High Transmit Power

LDPE - Low-density Polyethylene

LTP – Low Transmit Power

mPAP - Mean pulmonary artery pressure

PA – Phosphatidic acid

PAH - Pulmonary arterial hypertension

PFC – Perfluorocarbons

PIP – Phosphatidylinositol phosphate

PSD – Power Spectrum Density

SF_6 - Sulphur hexafluoride

SNR - Signal-to-noise ratio

UCA's - Ultrasound contrast agents

US – Ultrasounds

1. Abstract

Nowadays, many diseases provoke clots at unique or different points of the cardiovascular system. Sometimes these points where clots are generated cannot be accessible for surgeons since they present complexity at the time of interventions, as it occurs for instance, inside the brain or some other parts.

These coagulations occur from preventing an excessive bleeding when vessels are injured. Proteins present in blood, will form a scaffold-like structure letting after platelets join it, in such a way it changes to a gel state. Normally, this clot is dissolved by the own organism once the blood vessel is healed. These clots usually occur in arteries or veins. These two kinds of vessels work together in carrying blood throughout all the cardiovascular system, but each one works differently, since they have different functionalities.

Arteries, by definition, are those blood vessels which exit the heart in order to deliver blood to the whole body. Nevertheless, usually they carry oxygen-rich blood throughout the body. Due to all arteries exit the heart, they have strong walls which permit them supporting the high pressures created by the heart pump.

In the other hand, veins are low-pressure vessels which all of them end at the heart. They usually deliver deoxygenated blood from the organs back to the heart. Therefore, when a clot is formed in a vein, could difficult the blood flow while returning to the heart provoking symptoms such as swelling or pain. An immobile clot normally does not become dangerous, but sometimes it breaks into several fractures which travel to a smaller vessel, collapsing it and preventing adequate blood flow.

One of the biggest cardiovascular problems that can be caused by these, is DVT (Deep Vein Thrombosis), which is a kind of clot usually presented in large extremity's veins such as in the femoral vein. These clots sometimes detached from their original place where they have been formed, traveling throughout the body until they reach the lungs, in where these clots become wedged and provoking a pulmonary embolism, being extremely dangerous.

New techniques such as UCAs (Ultrasound Contrast Agents) are being introduced to the field, consists in injecting through the bloodstream microbubbles in order to help imaging via ultrasounds. When these bubbles oscillate at its characteristic frequency performing harmonic resonance, pressure can be calculated at certain points in the circulatory system. This characteristic frequency is the result of insonating them with a certain pressure pulse. Regarding on the area of the body the bubble is located and the pressure, the resonant frequency will change.

2. Introduction

2.1. Motivation

Ultrasound contrast agents

Since the very first time ultrasounds medical applications were discovered in the decade of 1960's, have been used in many scenarios providing real time diagnostic images. Also, something that has made these ultrasounds so popular is its ability of being non-harmful to the body since they are not based in emitting ionization energy pulses as well as its non-invasive procedures of obtaining the images.

Ultrasound devices are based on the differences that tissues have in terms of acoustic impedance. In other words, the principle behind is the same principle that let bats orientate them in the environment by emitting ultrasounds. When these ultrasounds bump into a stone wall for instance, US rebounds like an echo and bats receive it locating them in their environment.

With a piezoelectric transducer, which transforms electricity into mechanical energy, ultrasonic waves are emitted and sent through tissues. Once these waves are sent, they rebound with scattered amplitude depending on the acoustic impedance of tissues which is measured by a receptor. This amplitude, is usually proportional to the difference of acoustic impedance between tissues, and in such a way, permits the creation of the image. Normally, the receptor is the main piezoelectric device, since it has also the ability of transduce mechanical energy into electric energy.

Although these devices seem to be very efficient, actually the quality obtained by them is low due to noise and need the enhancement of ultrasound agents which let this imaging system device to gain quality leading for a proper diagnosis.

Ultrasounds Contrast Agents, also known as UCA´s, are molecules formed by colloids which are arranged in micro bubbles when in blood flow due its usual coating formed by phospholipids which makes them form micelles. Their coating is usually composed by a biocompatible phospholipid and a low solubility high weight gas which is filled into the micelle. Their diameter has to be smaller than cells in order to let them continue the blood flow and prevent the filtered of the lungs. Therefore, the commercial Ultrasounds Contrast Agents have diameters from 3 up to 10 µm.

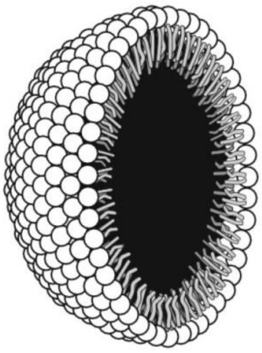

Figure 1: Micro bubble with a phospholipid coating [2]

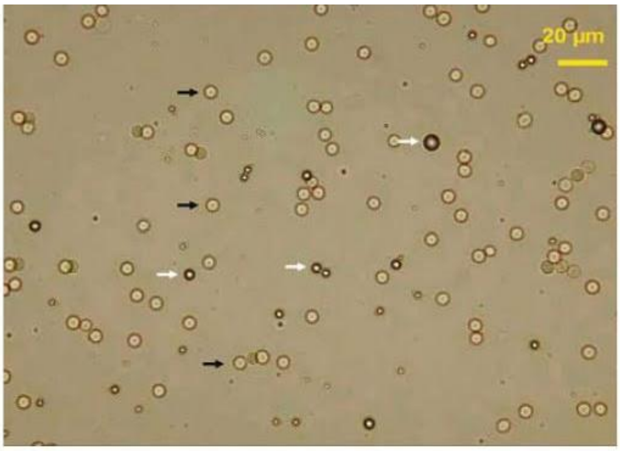

Figure 2: Snapshot of a microscopic scenario in which Ultrasound Contrast Agents (white arrows) are compared with RBC's (black arrows) [3].

The reason this Contrast Agents have a shell when in its dispersed phase is for avoiding some complications that will prevent them for performing its duty, these complications can be such as gas diffusion or even bubble merging making bubbles increasing its volume and generating problems like being filtered on the lungs, preventing changes in the gas volume or even provoking the micro bubble collapse by increasing the energy threshold needed for two bubbles becoming one.[4].

As these micro bubbles are usually stable, with the properties described in the paragraph from above, UCA´s have been consolidated as an update for ultrasound imaging, obtaining a better image quality as well as higher safety for the patients [5, 6]. Although there are different types of shells regarding the chosen application, then the coating composition can change, changing its thickness, stiffness or other properties.

These differences can be appreciated depending on the biocompatible coating used for UCA´s, which mainly depends on the brand and its application, some examples of them are shown in the table 1

Table 1: List of commercial UCA's and their composition [7].

Name	Shell composition	Gas	Manufacturer
AI-700	Polymer	Perfluorocarbon	Acusphere
biSphere	Gelatin/polymer	Air	Point Biomedical
BR14	Phospholipid	Perfluorobutane	Bracco Diagnostics
BY 963	Lipid	Air	Byk-Gulden
Levovist	Galactose/palmitic acid	Air	Schering
Definity	Lipid	Perfluoropropane	Bristol-Myers Squibb
Imagent	Surfactant	Perfluorocarbon	Imcor Pharmaceuticals
Optison	Albumin	Perfluoropropane	GE Healthcare
Sonazoid	Lipid	Perfluorobutane	GE Healthcare
SonoVue	Surfactant	SF6	Bracco Diagnostics
MRX-408	Lipid/ligand oligopeptide	Perfluoropropane	ImaRx
Quantison	Albumin	Air	Andaris Ltd
QFX	Albumin	Perfluorocarbon	Guangzhou Nanfang Hospital

In 1968, Gramiak and its team noticed when saline solution was injected in big sized blood vessels such as the femoral artery, an increase of the scattered ultrasound was perceived. Therefore, they discovered by first time the use of UCA´s.[8]. This saline solution, when injected became in dispersed phase making increase the resolution of the images obtained than the ones performed before without the saline injection. They notice that micro bubbles increased the differences between acoustic impedances of tissues, therefore enhancing image quality, causing a huge scattering of the ultrasound increased by the UCA´s as it can be seen in Figure 1. These contrast agents were used by scientist until they overcame with the problem of micro bubble merging and collapsing. Therefore, they decided to encapsulate them, leading for two different approaches. The first one was the creation of claddings for the micro bubbles in order to prevent the complications described in the previous paragraphs. This claddings were at first composed of proteins [9], lipids [10] which clearly can be appreciated in Figure 2, or even sugars [11], substances which the main organism should accept and even metabolize. Also, sometimes, Gramiak and its team, substitute the encapsulated gas to another with a less soluble gas leading to best results, such as sulfur hexafluoride (SF_6) [12] and perfluorocarbons (PFC)

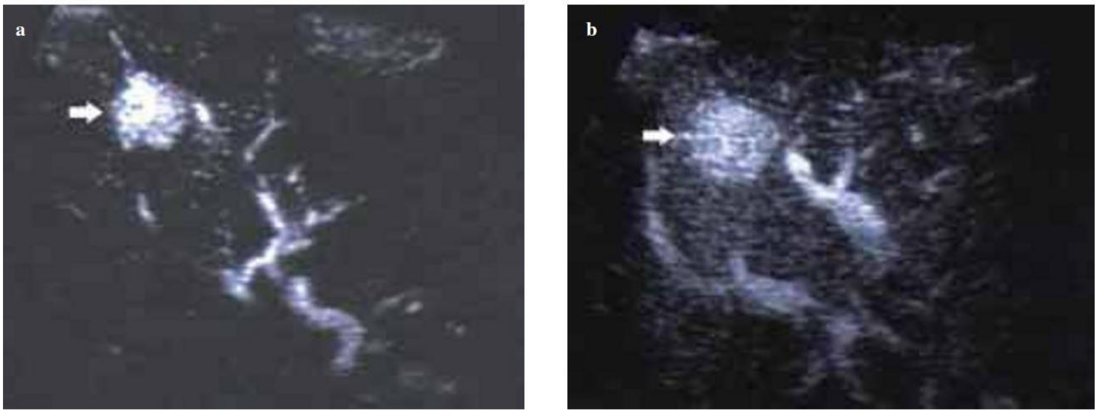

Figure 3: Images of a hepatic adenoma obtained using ePHD US technique in (a) arterial, (b) portal and (c) in late stage [13]

These scientists patented a contrast agent that contained a shell which practically blood insoluble and its gas is also non soluble through its shell. Although the perfect model would be a UCA with these properties working perfectly, the contrast agents Gramiak and its team proposed had some limitations. The gas they used was Sulfur Hexafluoride instead of the air bubble which was used before and a coating made of phospholipids due to its low diffusion and hydrophobic properties. This Sulfur Hexafluoride worked as they expected and it is currently one of the gases most used for this application. The gas is later expelled from the body via lungs and the shell is usually metabolized by the body organs such as the kidney or the liver. [14], [16].

This gas can pass through a different phase before being degraded, because of the adhesion of these bubbles to the spleen and to the liver, carried out by the intake of the gas by white blood cells. Once this effect happens, it can be observed by ultrasound the presence of WBC´s whom have been ingested these non-soluble and low diffusion gases. [17-21].

Regarding of how the UCA is composed chemically, the agent would have a different acoustic response; this is known as the continuous oscillation of the bubbles due to some acoustic waves colliding at the bubble resonance frequency [12]. This reaction was noticed from Gramiak's group for first time when they injected several types a UCA solution in a patient throughout different days. At some point they realised, that depending whether the composition of the UCA, the acoustic response was different than the one obtained of a UCA with different composition. They also observed a low toxicity of the agents when used in vivo in humans. No UCA provoke an immune response of the body when neither injected nor metabolized.

When being used in humans, UCA's show a very good safety profile and a lack of renal, liver or cerebral toxicity [22]. The rarely observed adverse reactions are usually soft and transitory [22, 23].

2.2. Bubble’s harmonics

Subharmonics

When an acoustic wave bumps into a bubble, this last one will become an oscillator. Due to this oscillations of the bubbles when insonated, the ratio Power Spectral Density will show variants according the showed before. In this case, peaks will be present on it, both, at its harmonics and on its subharmonics. These subharmonics are defined as the half value of the frequency at which these bubbles resonances, and in 1999, Shi discovered a way to find the value of the ambient pressure using the main amplitude of the subharmonics [24], as it will be exposed later on the problem discussion, these scientific discovered a linear variation of the subharmonics when the amplitude versus the pressure are plotted. Thanks to this, Shi and Forsberg were able to measure the pressure in several situations. First the successfully performed it within its laboratory, later on, they could obtained this in every situation. This was took into advantage for applications such as the petrol industry, in which this way of measuring the pressure using the peaks which the subharmonics perform is used in order to obtain the points in which is better to extract a gas or a petrol well. The other main application in which this thesis will focus on is the medical application. This application, consists on introducing Ultrasound Contrast Agents, and as they are introduced as micro bubbles, uses the same procedure in order to obtain and calculate the pressure in a non-invasive way. By the way, pressure changes can be also measured by studying the change of this peaks in which we have discussed before. These changes occur due to energy changes when the bubble is insonated because of an acoustic wave.

A few years ago, two experiments were performed by two scientific groups. One of them was carried out by Andersen and Jensen on 2010, in which the first noticed that some peaks in the energy of these subharmonics were reflexed in the variation of pressure. As expected, they concluded that both, the energy and the pressure changes follow linear correspondence. Then Halldorsdottir in 2011 in which the project was based in the study of this procedure in in vivo experiments, discovering an application for medical imaging to the experiment performed by Shi and Forsberg in 1999 and what they discovered. This application, as said before, is totally non-invasive and non-harmful [25]. Both papers written by the two investigators groups, came to the conclusion that the energy peaks do not vary very much according to pressure changes, although pressure can be measured anyway. Therefore, they also needed to take care about some parameters such as the bubble concentration, the energy of the acoustic wave when emitted. This result was also obtained 10 years before bay Shi's paper. Since these three conclusions, Andersen and Jensen conclude that despite what they previously thought, there is not a way, at least now, to predict the acoustic pressure at which the wave will insonate the bubble. [24-27]. In order to explore more about this topic, Adam and Ganor studied exhaustively the theoretical background of the many possibilities of obtaining by the use of subharmonics the ambient pressure. They came at the conclusion that using both, harmonics and subharmonics, with a nonlinear bubble model, were useful for obtaining the later calculations of the pressure. [28], [29]. These researchers performed an experiment in which evaluated and insonated two different bubbles with different pulses. The medium on where the bubbles where lied had different concentrations of phospholipids. They noticed that higher concentrations of these phospholipids that simulated the blood environment, the bubble resonating frequency exhibited a peak that was higher as the concentration raised. This is because of the reason that phospholipids join themselves into micelles when they are founded in a hydrophilic environment. These micelles behaved as bubbles, emitting also resonating frequencies that affected the results obtained in Figure4.

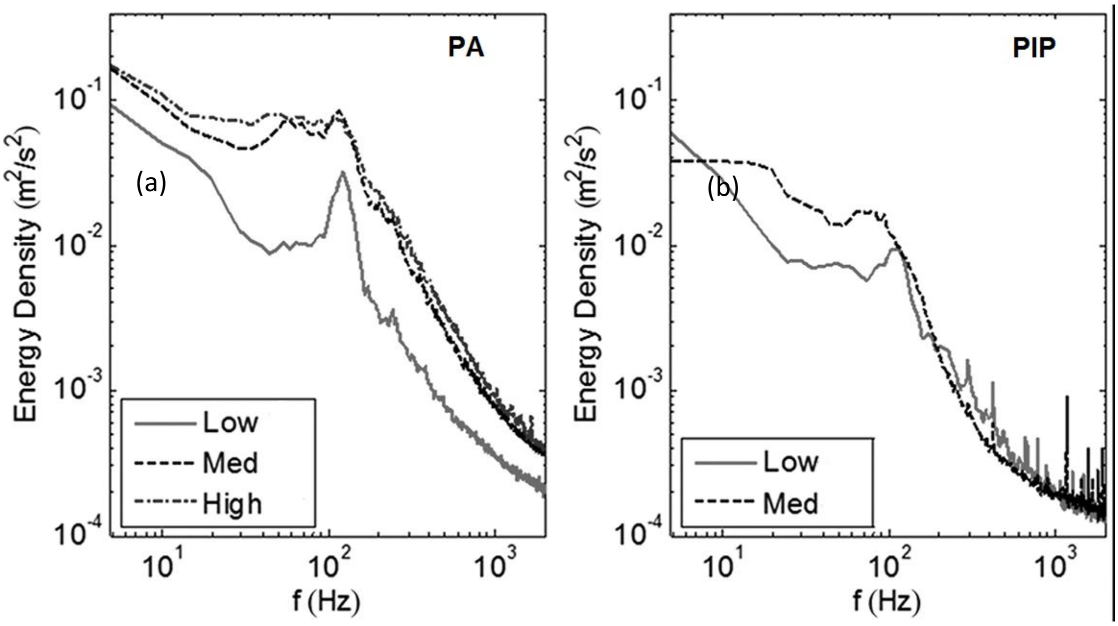

Figure 4: Energy Density plotted against the resonance frequency of UCA's coated by a) PA with low, medium and high concentrations and b) PIP with only low and medium concentrations. [29]

As the previous paragraphs say, these results successfully obtained their aim of obtaining the external pressure of the medium, by the introduction of some parameters that were needed for these calculations, such as the bubble concentrations. These parameters were useful for obtaining and predicting both, ambient and acoustic pressure. In the other hand, as these experiments took the parameters and obtained the results theoretically, it did not contemplate some other factors as other pressures or even external sounds, the algorithm was very limited since this factors affected to the energy and amplitude of the harmonics and subharmonics. Therefore, although the yields of the experiments were good, they were not as useful as expected, since its limitations in real life situations.

Resonance frequency

Previously than all the projects discussed before in 1933 Minnaert [30] and a group of researches started to focus on this area of the bubbles regarding fluid mechanics. They firstly discovered the oscillating behaviour of the bubbles when pulses bump into them, and also the relationship between the resonating frequencies of these bubbles and the ambient pressure. Thus, by measuring the scattered resonated frequency the ambient pressure and the pressure at which the bubble is located could be calculated. This happens because a direct relationship between the frequencies at which the bubble resonates and is insonated and the square root of the pressures. Later on, another group of researchers leaded by Fairbank [31], focused on the different scenarios of insonating a bubble, obtaining three main results. This results were the ones explained before, collapsing of the bubble, non-linear oscillations and linear oscillations. [30]

These studies were used and improved by some other researchers in the early 2000 in order to find and calculate pressures by previously knowing and using the frequencies at which these experiment's bubbles resonate, its harmonics and subharmonics. Therefore, for this reasons, some researchers such as Bouakaz, Leighton or Adham that will be mentioned in the next sections, used them for their experiments. [32-35]

Bouakaz and its team used the Fairbank experiment as a premise and continued striving for making a more complete study, and obtaining more accurate results. The aim was to study the properties of the bubbles when immersed in different mediums. As the pressure changes according to the density of the medium, considering same volume and conditions, when those bubbles were immersed in water, as the pressure was a bit higher, the scattered frequencies obtained were different. Surprisingly, Minnaert, 50 years before explained this situation by recording bubbles responses to pulses. [30]

As the pressure was obtained by calculating it from the resonance frequency of the bubble, the aim of the next experiments such as the one performed by Aldham, in

2010, was to obtain more accurate results.[34] Therefore, Aldham, changed the Minnaert's experiment by placing the bubbles in a plate. They formed the problem by taking into account many adverse conditions such as the effect of this plate into the insonating bubbles. They also noticed the ambient pressure is proportional to the resonance frequency of the bubbles, since small variations of the pressure, varied the resonance frequency. Because of this, they could measure little variations of the resonance frequency of the insonated bubbles and calculate also changes of the pressure of the order of 1Pa. The problem that they did not manage was the size of these bubbles. These, sized 3mm which were bigger than the bubbles found in nature. Since the experiment, was not as realistic as it was expected, the results could not be used into account for obtaining the pressures inn which bubbles are located inside the body. Then, by using UCA's the properties of the experiment changed, and they discovered a few years later, that the frequencies obtained, were much higher, even a 50 per cent bigger than the previous ones obtained without using the UCA's technique. [36]

Bubble shrinking

In 1993, Jong and its scientists showed that there was dependence between the shrinking radius value and the time when this bubbles are immersed into a stagnant fluid. This radius diminished at a certain rate which depends mainly on other parameters such as the external pressure. From this experiment we can get the conclusion that the shrinking radius rate depends on the external pressure [37].

As discussed in the previous sections, in 1999, another group of investigators, involved by Bouakaz .They introduced to the experiments UCA's, which these micro bubbles were insonated by short but very high intensity waves. These pulses vanished the phospholipid coating, although it also prevents the inner gas to diffuse or dissolve into the medium. Once this happened, they measured the time that lapsed between the coat diminished and the total diffusion of the inner gas [33].

Later on, on 2004, Postema continued the De Jong's experiment obtaining more detailed numerical solutions as the problem was formed much better. This experiment established a study with diverse bubble compounds, in order to make a more suitable and detailed field studio. This group of scientists obtained a better resolution results in comparison with the shorted time lapse of the bubble gas shrinking. This time lapses by the order of 30ms, which was really difficult to measure in those days, regardless the results obtained. For other researchers, this timing was a huge problem to solve, since the period was much smaller than the heart rate. The main problem that these scientists managed with was to obtain still a good resolution in in-vivo experiments, even when the cardiac cycle comes in. During the heart rate occurs, some muscular noise appears due to the contraction of the cardiac muscle and also because the propagation of the impulse by the Purkinje cells. Both scenarios interfered in the experiment by affecting the amplitude of the acoustic pulse. It is also useful to say, systole and diastole are carried out in times of 50 ms also, therefore, the problem dealt was considered. Since this problem was a headache for Postema and its team, they solved it by using Ultrasound Contrast Agents [35, 37].

It is useful to be taken into account is what happens when the fluid is not into a stagnant state. In this scenario, other parameters have to be concerned, such as the gradient velocity or even the steady and unsteady terms which can be easily obtained and calculated by using Navier Stokes equations. [32, 38, 39] On the other hand, although these terms are easily calculated, the main problem is much difficult to be formed and also to be accurately solved. Thus, some medical applications, for instance the measure of the inside pressure of the heart were difficult to carry out and to solve. Since inside the heart, a blood flow is constantly moving through it, there are velocity gradients in all points inside the heart. As we previously have said before, Postema and its group bumped into the problem of solving it when the fluid is not within stagnant state conditions. Since the bubble's radius shrinks as the time passes, it makes less accurate to measure the time of the bubble diffusion, and this problem grows when a negative pressure intercedes into the system [35]. Thus plotting the radius against time, we would see a final plateau reaching and tending to zero, therefore, it can be a bit imprecise to predict the time.

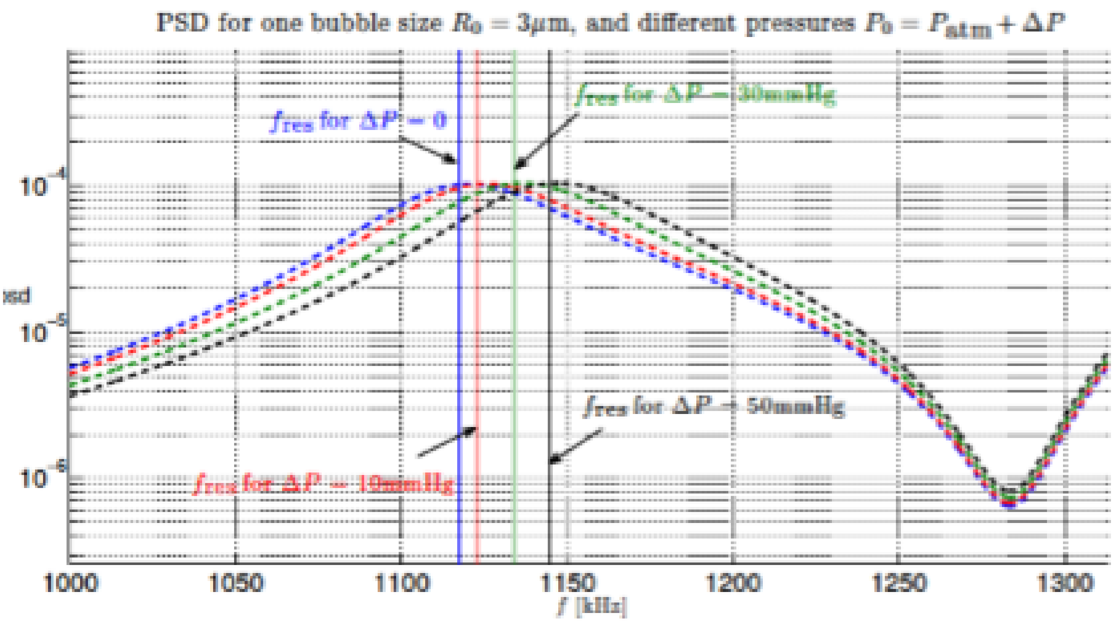

Figure 5: Plot which represents how the frequency of the bubbles vary according to pressure changes [40]

Bubbles as linear oscillators

When a wave, such an acoustic one with energy and a wavelength which exceeds a bubble's diameter, and collides with it, will create a pulse that later will make the bubble become in an excitation phase causing the bubble to oscillate. These oscillations can be divided in three main types depending of the nature of the main wave that provokes the bubble to become excited. We can determine the type of oscillation, since this one, depends on the amplitude pressure that the wave exerts to the medium. [41]

The first possible scenario contains a bubble collapse caused of a very fast oscillation. The second one occurs when the bubble oscillates a little slower causing it to oscillate following a random pattern and the last scenario occurs when the oscillation velocity is dropped down, becoming a slower oscillation than the previous scenario. When this last option occurs, the bubble will oscillate following a linearly known pulse making it to stay in its own equilibrium.

The oscillation that a bubble follows also depends in the pressure that the acoustic wave exerts in the environment. The bubble will contract when the pressure is bigger than 0 and when it is less than it, it will expand throughout its radius. Therefore, as waves are produced and travelled one by one like sea waves, between each wave there are moments of negative pressure in which the bubble expands until the next wave bumps into with the bubble making it to contract, and so on, causing a periodic oscillation that will take place until the last wave comes to scene, causing one last contraction and after it an expansion that will be followed to an equilibrium phase in which the bubble remains stable. This equilibrium is reached when the main medium in which the bubble is immersed overcomes the drag force that the wave was exerting.

The oscillation phase diagram has many similitudes to a sinusoidal movement, in case this oscillations that are taking place are linear, which it means that the movements that the bubble performs, are mainly periodic, as it can be predictable. This can be perfect appreciating in Figure 6 a).

It also can be noticed the radius change, in the oscillation phase as the bubble is periodically contracting and expensing (which denotes a growth and a diminish of the bubble radius)

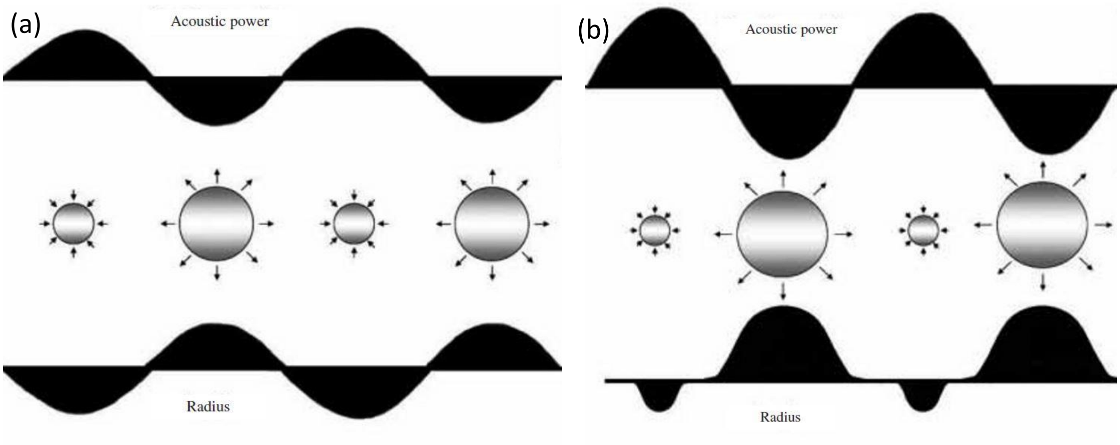

Figure 6: Bubble oscillations when insonated by a pulse. a) Represents linear behavior of the oscillations while b) non-linear behavior. [13]

As it was described previously, the difference between the growth and shrinking of the bubble resides in the wave exerted and also the bubble properties. This can be resumed in a single value, obtained as a ratio of the difference of the radius. This radius can be divided in two main types regarding the type of oscillation described by the bubble, as the third one (when the bubble collapses, no oscillation can be performed by the bubble). When the bubble performs a linear oscillation, the harmonic components of it, are the sinusoidal movement described previously and showed in Figure 6 b).

On the other hand, when a bubble describes a non-linear oscillation, the scenario changes. These oscillations turn into non-periodic since they are not followed by a certain pattern, therefore they can be said as an asynchronous harmonic resonance. Due for this reason, the bubble emits a response which is not only its fundamental one, as it happens in linear oscillations, it also emits another responses out of its resonance frequency which usually are multiples (ultraharmonics) or compounds of it (subharmonics). Both of them are described in the Figure7 presented below.

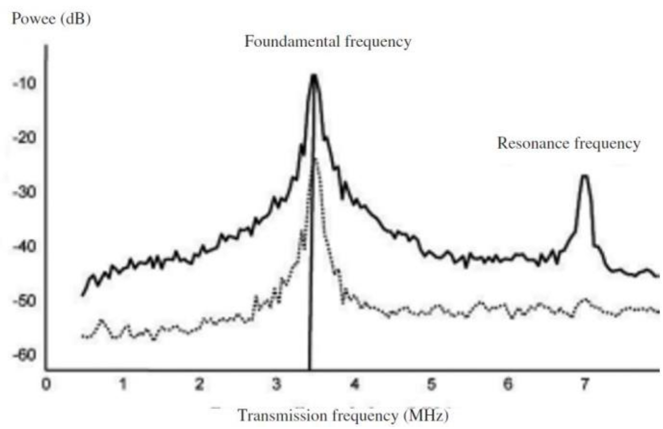

Figure 7: SF_6- Spectra performed according the Power spectral density. Black line corresponds to non-linear oscillations while the grey line corresponds to the Linear response of the SF_6-

bubbles. [42].

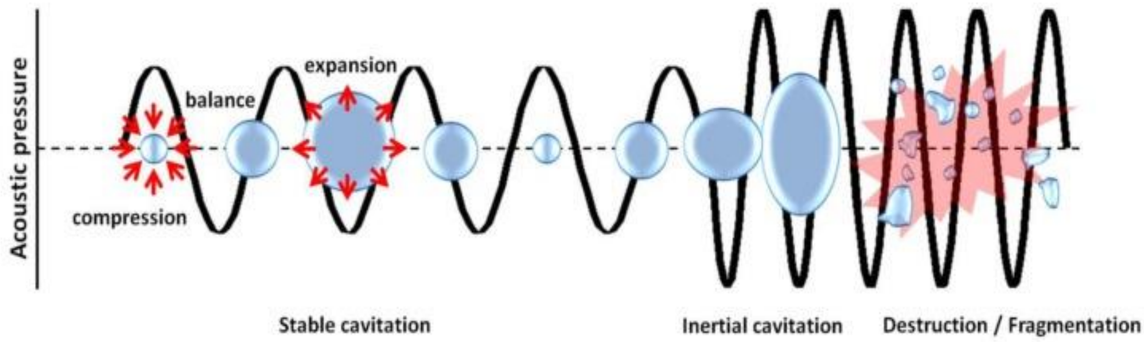

Figure 8: Bubble undergoing through cavitation when the frequency exerted reaches its threshold. [43].

The frequency, at which the acoustic response of the bubble is emitted, was described by Prosperetti. He noticed this frequency depended on the medium, the bubble's diameter and the environmental pressure, and characterized by omega sub-zero. [30, 44]

$$\omega_0 = \sqrt{3\gamma P_0/\rho R_0^2}$$

According to the equation above, ρ corresponds to the density of the system, R_0 determines the initial bubble's radius when in equilibrium and before being insonated, the same definition comprehends to P_0 but instead of being the radius, regards about the initial pressure. Finally, γ is the term which indicates the polytrophic index within the system. [45]

2.3. Introduction to US Techniques

Ultrasound techniques and UCA's usual applications in diagnosis

Because of the reasons explained before, when a bubble is covered with a UCA, its resonating frequency rises up at to a 50% more than its normal frequency when insonated. Once the pulse reaches the bubble's medium and the bubble, they will oscillate at its resonance frequency and behaving in three main ways, as discussed. But one thing has not been mentioned nor discussed, is these bubbles have two ways of becoming excited once the pulse bumps into them. The first one is called High-Transmit-Power or HTP, and as the name who describe it says, the amplitude of the acoustic wave that will insonate the bubbles will be much greater, by the order of MPa, therefore, the micro bubbles will collapse immediately when insonated at such as great amplitude. This collapsing is observed by the wave response recording, in which it can be seen something that looks like an explosion.

As this modality has its aim in obtaining a very varied spectrum of acoustic responses, the micro bubbles will be coated by a very tiny and small covered that will not impede or difficult the collapsing of the bubble and its later explosion. These bubbles are also filled inside by air, thus increasing the acoustic response. Therefore the result, as expected will be characterized by its brief prolongation during time.

On the other hand we have the other model which is characterized because of its low amplitude signals, by the order of kPa. Therefore, due to these reasons, it will be named as Low-Transmit-Power or LTP. This LTP method provokes a nonlinear oscillating response, since the frequency of the pulse is higher than the resonating frequency at which the bubbles oscillate. Therefore the response will have in addition, more responses such as subharmonics that will lead to the nonlinear oscillating bubble's behaviour. These subharmonics can be appreciated well in the spectrogram of frequencies obtained by the transducer that records the bubble's response. But this response obtained is also short, although not as short as the one obtained when the bubble collapses by the High-Transmit-Power method. In order to make this time response bigger, the micro bubbles used are usually Ultrasound Contrast Agents with a hard shell made usually by phospholipids for remaining together in micelles and fulfilled with phosphor or fluorocarbons, which its response will be transduced into a better quality and time prolonged signals. Because of this time prolonged properties, Ultrasounds Contrast Agents are widely used in medical applications, since they solve many problems that the theoretical approaches of Aldham and Baukaz did in the earlier 2000's by improving Minneart's problem.[30, 33, 34] This happens because as the UCA's have denoted properties that increase the later resolution, the collapsing frequency threshold and time. As it's also known its composition, there are no needed calculations in relation to calculating neither the shear stress nor the viscosity, since its known from advice.

These methods with the help of Ultrasounds Contrast Agents have been really useful for the better blood flow study through the circulatory system. But this UCA's are used as well with some other ultrasounds techniques in order to make this possible by increasing the Signal to Noise ratio and taking into account the Doppler effect, thus increasing the imaging results an allowing to be not only used in the circulatory system, but for organs and tissues which are irrigated by the blood flow. In any case, these methods have artifacts limitations although newer techniques have reached very decent results by the introducing of recent methods that keeps the study clear from them.

So, some of the methods that are recently discovered and used will exposed by the classification performed by Quaia [21, 42, 46]:

1. Modulated Phase: These methods consist in the Low-Transmit-Power method by the use of many waves at the same time that will be scanned at once. Indeed, these waves have the same modulus but have different sign, therefore can consider them as inverted pulses. Due to this and the high Signal to Noise ratio, it is easy to eliminate from the transducer signal the components of these frequencies which are not desired to obtain. By the way, it is also possible to make a higher classification dividing this group into three smaller ones, characterized by having: one main emission of these sample of pulses, two emission and three.

2. Modulated Power: This technique lies in the emission of widely power pulses using the Low-Transmit-Power methods. The main difference between this technique and the previous is that this method uses a widely range of power signals instead of the widely range phased signals of the Modulated Phase. The responses are normally linear which is used for cancel the transduced signal, and the echoes produced are used for imaging.

3. Modulated Phase and Amplitude: Both, Modulated Power and Modulated Phase are used and joined together, with very good results.

4. Doppler based: These techniques rely in the previously discussed High-Transmit-Power by creating and emitting several pulses at once, therefore can perfectly characterize a wide range signals. The main difference of this technique and the Modulated Phase is the use of different power methods, Low-Transmit or High-Transmit-Power. It is widely used for tissue acoustic emission and detection.

5. Harmonic Guided: Taking into account the oscillating response that bubbles exhibit once they are insonated by a pulse which frequency is higher than the bubble's fundamental frequency. Therefore, this method resides on the nonlinear behaviour of the bubbles, by characterizing the harmonics and subharmonics of them. These components contain information about the oscillations performed by the bubbles. The main problem is the noise provoked by the surrounding tissues when it is used in in-vivo situations at High-Transmit-Power. Due to this, Low-Transmit-Power are widely used in order to avoid these tissue's noise.

Due to the researches that have improved this field of the fluid mechanics, the application field of Ultrasound Contrast Agents in medicine has been growing more and more in these last decades. This application has reached that far that even the results for tissue or organ imaging, such as the use of UCA’s in kidneys and liver (since are organs that are close to the outside) can be compared with the ones obtained by tomography or even of nuclear resonance. [46 - 49]

The uses of this enhancing imaging technique are really spread for many purposes. In the last five years, these techniques have been used for cirrhosis detection and also for thrombosis location. There are also some studies than proof the efficiency of UCA’s in liver transplantation, seizures, spleen haemorrhage, and even the detection of malignant tumour growth and its differentiation with the benign ones. Nowadays there are researches with successful results for monitoring tumours in internal areas using High-Transmit-Power in order to imaging them, such as the spleen tumours. [50 – 54]

These technique of combining Ultrasounds with Ultrasounds Contrast Agents is gaining importance in the cardio imaging field, by monitoring ventricles and auricles when for instance stents have been implanted or even it is possible to monitor the blood flow through shunts (in case these are present, since it’s a really unusual condition characterized by holes in the heart walls that separate the blood, provoking a mixing between the deoxy and oxygenated blood), for evaluating the cardiac tissue in case of myocarditis, monitoring for heart attack preventing or even the proper visualization of the crane encephalic blood flow. [55 - 58]

UCA's medical applications

As a continuation to the previous brief introduction, in this section, these applications of Ultrasound Contrast Agents will be totally explained. It is a fact that this techniques are developing and improving the field of medical imaging, and since these procedures and the devices needed, are totally non-harmful and low priced, it is said to be a competitor for other techniques in medical imaging that are much expensive such as the MRI or even potentially harmful, like the TC. As explained in the last part of the previous section, this study field of the fluid mechanics is yield to be more and more applications in many technological sectors such as the petrol sector in order to verify and calculate the pressure of the gas for extracting as well as where to locate the well for improving the extraction of the petrol and gas.

The other main application is the one introduced already and in which this part will focus, the medical application of UCA’s.

1. Drugs through blood flow: This idea relies in the transportation of drugs encapsulated in bubbles through the blood blow until reaching a certain

location in the body where are needed by the use of Ultrasounds in order to move those bubbles through the blood flow. This UCA's can be genetic vectors in which the genetic material is deposited inside the external shell or even can be attached at it. When the UCA's are at the desired area in the body, with a high pulse which frequency is much higher than the normal resonating frequency of the bubble, these collapse freeing their inner containing or the external shell with the attached drugs is free into the medium and the target cells will take it in. [43, 59- 61]

2. Medical ultrasound's imaging: This application lies in leaving the bubbles travel through the circulatory system. These bubbles have specific molecules attached at their shell that will bind target molecules when they reached the desired location. Usually they have high affinity to bind ligands that are present in diseases. The coating shell of these bubbles has usually certain fluorescence or a characteristic acoustic response for being detected easily when ultrasound imaging. This technique is really spread through disease diagnosis, for instance, for cancer detection, marked micro bubbles with high affinity at glucose are injected through the blood stream. Since cancer cells have a higher metabolism of glucose than no other normal human cell, these microbubbles bind the glucoses and if this is monitored, a high concentration of them will appear in the cancer location. In order of detecting cancer locations in the body, another technique used is to attach ate the coating shell of the micro bubble antibodies that will bind molecules produced by tumour cells from the endothelium. Normally, these molecules are P-selectin or alpha-integrin. These bubbles, as explained before, will change their response when attached at these molecules, letting the cancer diagnosis easier. Another application of this is the change of the acoustic response of the bubble when specific epitopes present in a certain disease bind the bubble, so when the bubbles do not bind these epitopes will have a normal response, and when they bind them, this response will vary making it perceptible at the transducer, which will make it more visible and clear to disease diagnostic by Ultrasounds. [59, 62 – 64]

3. Bubble collapsing: This application relies in the same principles used for point 1. Knowing from advance how the responses for insonating pulses of the bubbles are, it is possible to obtain a desired result when the conditions are the ones needed. Therefore, we can make bubbles collapse when high frequencies with also high-transmit-power method used to emit the pulses and make them insonate the bubbles. By using this principle, we can also make the bubbles, which were filled with anti-coagulant drugs previously and then introduced in vivo in the system by injecting them, travel using ultrasounds

and when these bubbles are close to a thrombus make them collapse and in such a way free their inner cargo that will make the thrombus dissolve into the bloodstream as it can be seen in Figure 9. [65, 66]

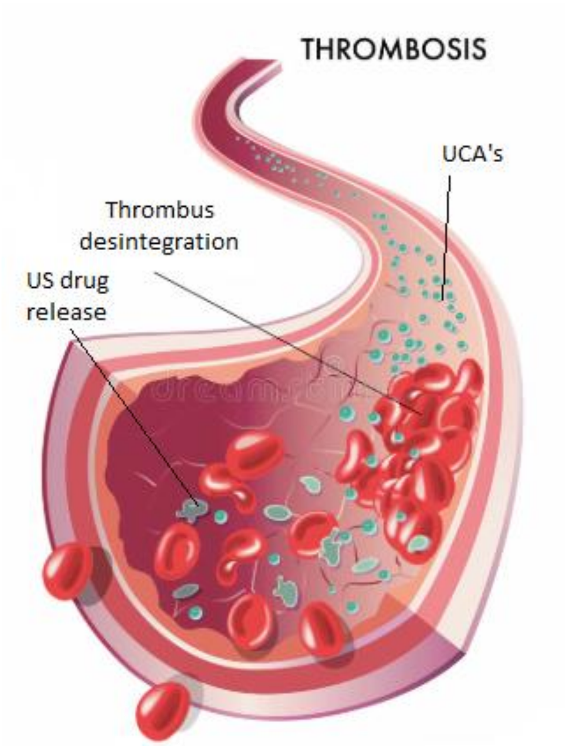

Figure 9: Thrombus disintegration using US drug release technique [66].

4. Pressure analysis: Since Minnaert and its group of researchers started to study and focus on this filed of the fluid mechanics, the pressure analysis has been a real duty and problem to solve. Since the very first moment, they noticed this aim will be an arduous task for be solve. Many researchers have focused on this solving partially in some punctual conditions such as the pressure measurement of Aldham [30, 34] that was only useful when these procedures take place inside a laboratory. Although this application relies on the procedure to obtain the pressure measurement inside a body without having to puncture or making an incision to the patient, therefore we are talking about non-invasive techniques for obtaining pressures in vivo. As mentioned in the previous sections, Fairbank [31] was the first scientific who proposed this innovative and original idea. Using Minnaert formulas, Fairbank observed changes at the bubble's frequency response as the pressure at which the bubble is surrounded changes too. Once this idea was took on mind, they knew for sure that, when obtaining the solution for the task of obtaining a procedure for obtaining the bubble's pressure by using their frequency response when insonated, would, definitely, solve many other problems such as this one in medicine. By changing the pressure in which these bubbles where located, they could perform a differentiated study of the acoustic

response regarding the changes of pressure within the system as it can be seen in Figure 5 of these same section. Therefore, it was possible to make a certain relationship between them, which corresponds to the square root of the harmonics of the acoustic response, which is related with the pressure change, and due to this, the change of the acoustic response was characterized. Nevertheless, this application has always have a theoretical background but none results when performed in real situations such as this one. There have not been convincement results for this to be considered as a therapy or technique. The efforts took into account have been numerous and even currently there are many researchers from all parts of the world contributing with projects and studies for solving the problem. The main achievement is when Ultrasound Contrast Agents are used, some results are yield. Although changes in the acoustic response have been detected, once these contrast agents are in the scene, pressure changes are usually much easier to be calculated since the properties of these Ultrasound Contrast Agents are previously known and used as wish. One of the most difficult task to solve is the pressure measurement of difficult access body areas, such as calculating the pressure inside the heart or at arteries, the most studied is the Aortal pressure measurement, which will discussed in the next section.

Difficult access pressure analysis.

Some areas within the body have very hardly and difficult access, this could be because these areas are located in the very intern part of the body and limit with many organs, or due to the high risk of provoking any damage to function needed organs. Because of these reasons, some areas like the brain, the heart or the beginning of arteries as the Aorta, are common and needed studies for being resolved. As there is no solution yet to the problem to non-invasive pressure measurement, the pressure at these areas has been a headache for doctor that need it for many health reasons. The most common reasons are heart failure and vascular diseases.

But this measurement has to deal with other field diseases, such as the pulmonary ones, where calculating the pulmonary artery pressure is needed, and has a huge relevance, since the diagnosis depends mainly on it such as the pulmonary arterial hypertension, which will lead to a reduce of the volume and the flow of the pulmonary artery, and therefore a decrease of the volume of the oxygenated blood, and not covering all the needs of the human body. This disease can also lead to blood intoxication by the increase and amount of carbon dioxide in blood. This carbon dioxide bounds the haemoglobin present in the red blood cells and intoxicating them and provoking hypercapnia. This mainly occurs due to a shrinking of the pulmonary artery radius making the pressure to rise up to more than 25 mmHg when in normal conditions this pressure oscillates from the 10 to 20 mmHg. Once the pressure reaches the 25 mmHg, a right ventricle overload occurs leading to hypercapnia and even to heart failure. This problem makes itself worse when at this time, unfortunately there is no treatment for solving this condition once it has been reached. For that reason, the early detection is so important. [67 – 70] By the moment, the only effective way to measure with a certain accurazy is by measure physicaly in the heart, therefore using an invasive way. Because, as explained before, there is only a way of measuring pressures without having to be physicaly at the local point, and it is using Ultrasound Contrast Agents, altough this measure is not precisse and does not take into consideration other side effects such as the heart muscle noise, or the purkinje cells noise while they transmit the heart pulse. Because of this reasons, currently, it is needed to access to the inner heart in order to obtain a correct and accurate measure of the heart pressure as in the case, the pressure we want to know is the pressure at the heart. In case we would want to calculate the pressure at the pulmonary artery for a Pulmonary Artery Hypertension diagnosis, it will be needed to invase the body and measure the pressure within the pulmonary artery. This invasive way makes the procedure much harder than it suppose to be. There are many risks when accessing at the inner heart, later infections, pain and malease. Because of these reasons, obtaining an effective method for obtaining this non invasive therapies for pressure measuring, are such as important, as well as because of the loads of money that could be saved avoiding interventions for obtaining it. Nevertheless, there are some nuveau techniques that allows the analysis of the pressure at the heart, by observing the ventricles wall movements using doppler theqniques and with the help of Ultrasound Contrast Agents, pressure can be calculated at these points. In any case, this have inconvenients such as this is an approach and pressure obtained is not as accurate as wished and this methods has to be improved if in any case they want to be effitiently used. [71]

Nowadays, there have been some recently approaches and studies that demonstrate the efficiency of UCA's in this field, allowing taking into account some diverse acoustic noises that affect the final result of the signal such as the heart muscle movement. These approaches are more accurate than the ones obtained with only Doppler ultrasound techniques, and would serve to the doctors as a great help and a earning in time and money for the doctors and patients, without causing neither pain nor risk situations. It is expected to be a great help to clinicians in other medical areas like drug release when this drugs have to be administrated too the patients according to their heart rate and the blood flow. Because of this reasons, we will focus on the next section on discussing a more detailed approach of these techniques.

2.4. Non-invasive Pressure Measurement: Thesis description.

Experiment dynamics:

In order to understand better the experiment, there is only a thing more to be explained and discussed previous to going ahead to the thesis experiment. It is mainly explained as the formation of bubbles when one or several forces are acting in a system. This is one thing that has to be taken into account since it is a really common and body present phenomena. Thus, this is not as important as the study of the dynamic of bubbles not only because of its late discover of medical or petrol application, but because it also comprehends the cavitation phenomena. Therefore, as bubbles behave when in fluids with a viscoelastic solid behaviour, this catch the attention of so many scientists and tried to explain bubble's oscillation, properties and behave. From the very beginning, Rayleight-Plesset equations were used in order to formulate the problem and therefore, to obtain a coherent solution for it. [38, 39]

The main purpose of this thesis is to find a theoretical solution for this problem and define the relationship of the pressure and the oscillation of bubbles when immersed in a gel like medium, since this medium will be an approach to the body medium in which Ultrasound Contrast Agents are found when in body. As explained before, this will be a theoretical approximation for obtaining the pressure at points that are not capable or difficult to measure using non-invasive methods. These experiments are performed since a very first moment using Hamaguchi and Prosperetti researches, which were deduced, in addition, by the use of Minnaert's equations for solving pressure from the resonating frequencies at which bubbles oscillate [30, 44, 72]. As said in these equations explained before, the pressure and the size of the microbubbles studied characterized the frequency at which the bubble's will resonate when insonated by an acoustic pulse. This frequency will have a peak which will correspond to the maximum amplitude of the oscillations. Then, every parameter of the equation and of the system can be calculated once this peak is calculated, other parameters such as the ambient pressure can be calculated. Therefore, for the purpose of this thesis, we will use Hamaguchi's equations, with and without the Voigt terms, in order to perform and find a more accurate solution to our task. [72]

The medium that was theoretically used was gelatin from porcine origin, since it has same properties as human one and the results obtained were successful. This gelatin, looks like in all cases and behaves as a soft human tissue. Other gelatin parameters which were theoretically used were the human gelatin, and then both results compared, indeed, there are no significant differences between them, since the medium is mostly the same in terms of behaviour.

For the acoustic modulation, in order to create a spectrum that arranges as many as frequencies possible, the pulse chosen was what commonly is called, a chirp. This chirp is in other words a wave that has a huge set of frequencies continuously emitted. Therefore, the same procedures were performed for other parameters and varying the Hamaguchi conditions that he and his team performed a couple of years ago. This theoretic scenario observes bubbles behaviour when chirps are emitted through them and when are insonated. The theoretical bubbles which were used were essentially Ultrasound Contrast Agents because their gain in acoustic response, which, as explained before, were up to 50% more than when these contrast agents are not used. The theoretical scenario was developed in such a way to be more accurate and to notice small changes in the pressure that could affect the system, provoking altered signals and responses. Because of this, the Ultrasound Contrast Agents used, were programmed with a tiny shell as well as elastic, since if this coating was used, the effects of small changes on the system such as the pressure will not affect as much to the bubbles. This behaviour of these tiny and elastic shelled ultrasound contrast agents is possible only when low powered pulses are emitted, with equal or lower frequencies than the resonating frequencies of the UCA's, since if the frequencies are higher, the bubbles would be more pronate to behave following a nonlinear oscillations or even collapsing and leading the system to failure. In this current decade, Hamaguchi studied the non-spherical oscillation of the bubbles, classifying them and also giving an exhaustive analysis of them. They also tell it was difficult to obtain that result since the frequency they used in order to insonate those bubbles, was something between the frequency needed for obtaining nonlinear oscillations and the collapsing of the bubbles. Therefore, it was a hardly difficult experiment to carry on since any external frequency could affect the system obtaining non desirable effects. Thus, Hamaguchi decided to apply lower frequencies than the theoretical calculations, since they prefer obtaining nonlinear oscillation and the applying the correct pressure and obtaining the results that represent the Figure 10 instead of making unintentionally explode the bubbles and rebuilding the system once again repeating the whole procedure.[72] What is mostly done in this cases, since the response sometimes is affected by the harmonics of the environment, and although it reduces the accuracy of the information, a high pass filter is needed if the result has been carried away using the second components of the response, or a low pass filter if the result is obtained using subharmonics, in order to avoid those undesired effects nor harmonics that affects the main frequency components of the acoustic response of the bubbles, represented in Figure 10.

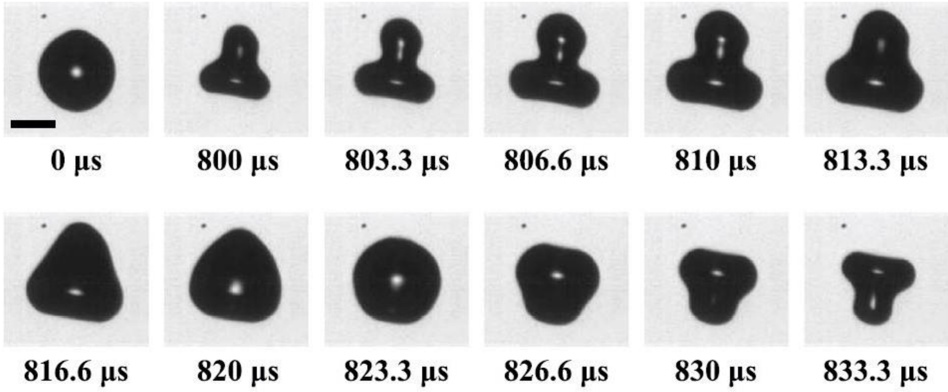

Figure 10: Non-spherical oscillations which its scale bar is 100 µm long.[72].

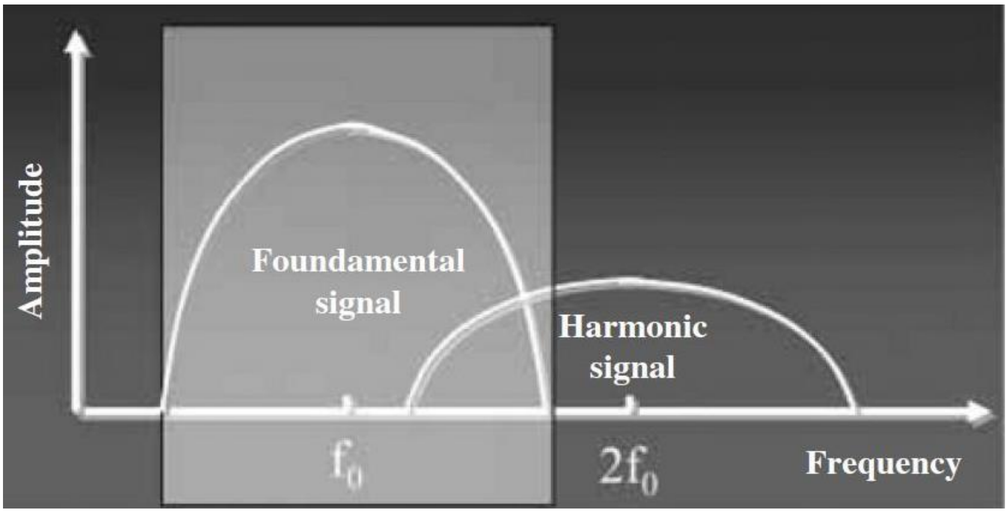

Figure 11: Overlapping of the response signal with the second harmonic represented schematically [46].

It is a fact that this thesis can be performed and successfully done by any researcher with the programs and the correct knowledge needed. But in order of being calculated and the results obtained, it was needed some years of researcher's work, such as the papers written by Hamaguchi and Prosperetti [44, 72], which were necessary for obtaining the results and carry on this thesis. Firstly, these studies obtained the equations needed to start and conceive the problem of analysing the pressure and the frequency of the system using Fourier analysis and making a theoretical scenario which allows to obtain a solution regarding the problem of the pressure discussed in the previous sections. Then, due to the knowledge in solving differential equations, we were capable of developing the original equations obtained from Hamaguchi and Prosperetti, with and without Voigt terms. This Voigt term comes from a symmetric tensor which order has been reduced. In this case, this term will be precisely explained in the experiment section. We will also use an algorithm characterized by using technique of extended pure harmonic detection or ePHD. This technique is a unique technique in which the harmonics are precisely detected and characterized by using other methods that simulate the low or high transmit power techniques as previously were seen. One aspect that makes ultrasound contrast agents a good decision of being chosen is due to the amplitude obtained in the response when these bubbles are theoretically placed in the system. Although this amplitude depends also in other parameters such as the bubble's radius, calculations will be performed for several cases such as bubbles with 200 µm, 60 µm and 120 µm, obtaining its corresponding graphics that will be later shown and results commented.

As previously said this thesis has focused really on the theoretical part of the problem, aiming for a later experiment that resolute and verifies the thesis. Therefore, the theory needed will be purely fluid-mechanics physics, in which everything could be computed from scratch by the knowledge of the Rayleight-Plesset equations. Once this is calculated, a medium has to be chosen, and since in this case we are trying to solve a medical problem, the application that it would lately have is within the body, in in vivo situations, therefore the medium chosen as said before was gelatin, both, human and porcine, to see the differences, which were mainly the same, demonstrating again the similarity of porcine and human tissues. By the way, it was gelatine because its properties such as the viscosity and the pressure exerted to the bubbles, seemed to be the same properties that soft tissues have when in vivo situations as Solano-Altamirano describes in its approaches, Table 2 [73] , therefore, it was a great decision.

Table 2. Shear modulus for different soft materials [73].

Material	G (atm)
Gelatin solution	0.083–0.434
Gelatin solution	0.0002–0.0004
Gelatin/agar	0.07
Neural retina	$\sim 9.87 \times 10^{-4}$
Liver	0.001–0.003
Liver (bovine)	0.10
Liver	0.001
Heart	0.001
Fat (porcine)	0.46
Breast (turkey)	0.10
Limb	0.01
Muscle	0.005–0.010
Articular cartilage	0.33–5.26
Knee cartilage	2.0–4.0

Another reason is although we could have computed this in the most theoretical way, letting be the bubbles suspended in vacuum, we tough that a more realistic approach to the system would be better in order to be continued in other experiments. Nevertheless, as Hamaguchi's experiments and equations are for bubbles which remain fixed in a certain location [72], our model will follow this conditions. Regarding the frequency used, the theoretical system will be composed of a pulse emitted which will have a wide range of frequencies, also called chirp, as explained. This chirp is the acoustic wave that wills insonate the bubble, provoking an acoustic response making the oscillation behaviour of the bubble happens. It is necessary to be taken on mind that at the time this experiment is performed in real time and space systems, the number of experiments needed to be accurate and take some relevancy, has to be a huge amount of them. Therefore, in order to the bubble to be characterized as it has to be, and understand and make a great approach of this procedure experimentally, lots of efforts will be needed, and in case this is performed by qualified personal, an economical compensation will to be considered, since the number of time dedicated would be much. This huge number of experiments and time is needed since there will be variations according to several factors that could be form even the weather (due to their environmental pressure), because of the infiltration of the gelatin with water making bubbles to move, or to the human errors. Thus, these variations have to be analysed and then a mean has to be performed according to the results if a coherent result wants to be obtained. In the other hand, chirps have a great relevance in medical scenarios, because of the technique used, they are usually very efficient methods in which it is possible to perform many measurements in short time conditions. And as explained before, the chirp is composed by a wide range of frequencies emitted at once and due to this, it is possible to obtain the acoustic response of the bubbles even though this was known previously.

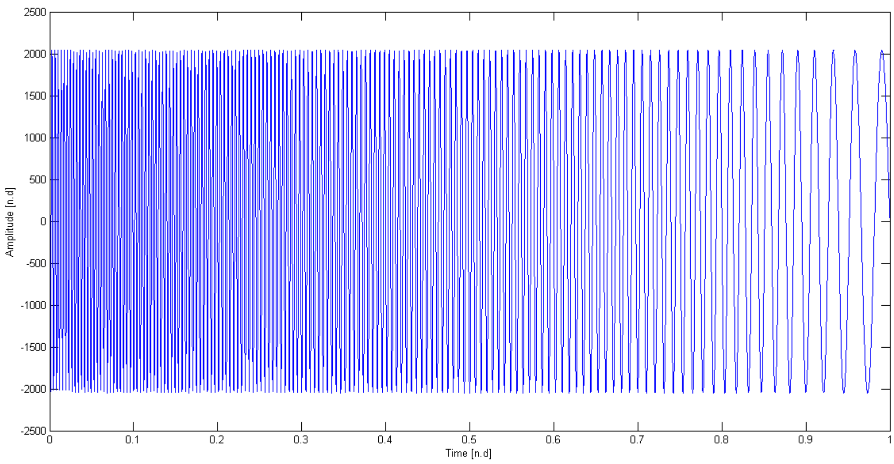

Figure 12: One of the chirps used by Judith Cueto Fernández on her thesis. [45]

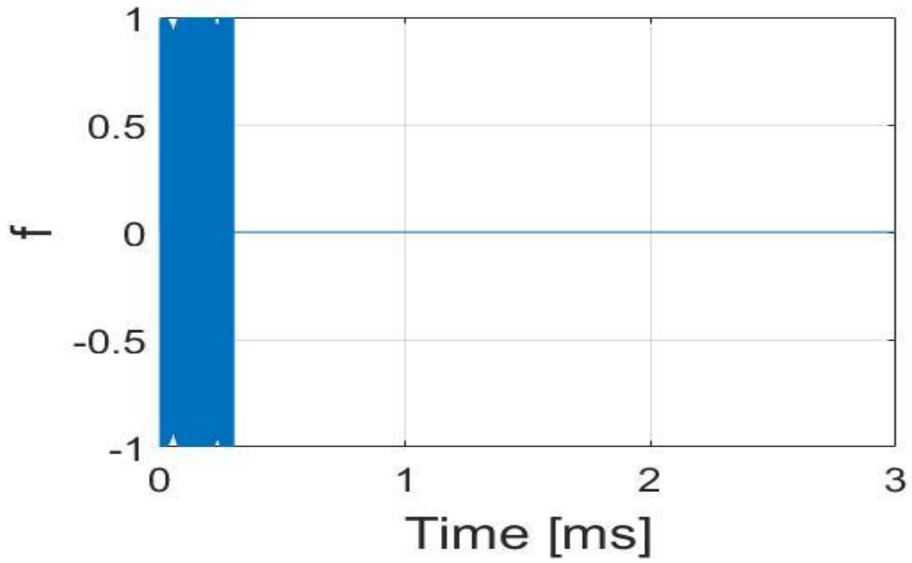

Figure 13: An example of one of the chirps used for this thesis. Its frequency range goes from 0 Hz to 1 kHz.

3. Theoretical Background

3.1. The Rayleigh-Plesset equation

Rayleigh [39] in 1917 and the later modification of Plesset [38] described the behaviour of the bubbles and their dynamics formulating an equation when these bubbles are suspended or immersed in a gel or a liquid medium. The equation is known as the Rayleigh-Plesset equation and this scenario is the one below, which is represented in Figure 14. Spherical symmetry is assumed as well the bubble is immersed in an infinite medium.

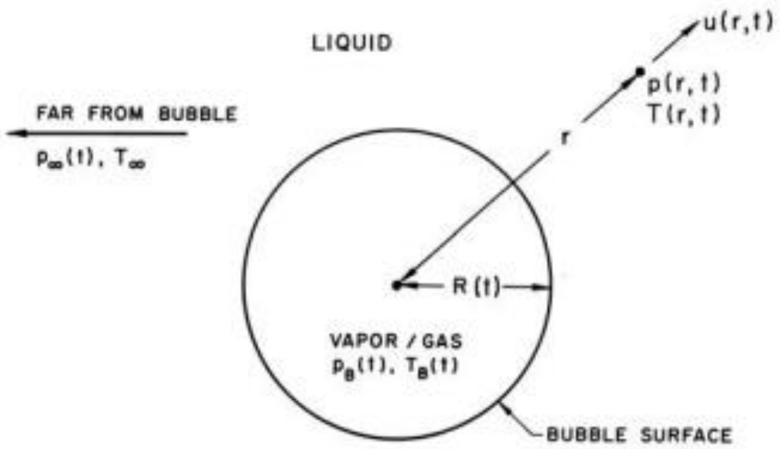

Figure 14: The Rayleigh-Plesset bubble [74]

It is also needed to take into account and explain that in the equation, ν determines the liquid kinematic viscosity, the term S relies on the surface tension of the bubble, ρ stands for the viscosity of the medium. Then there are only two terms that have to be referenced, which are the P_B that is the pressure at a point which is said in Figure 14 andP_∞, which stands for the acoustic pressure. Therefore, R will correspond to the radius, in which R is the original radius and dR the differential radius regarding time.

$$\frac{p_B(t) - p_\infty(t)}{\rho_L} = R\frac{d^2R}{dt^2} + \frac{3}{2}\left(\frac{dR}{dt}\right)^2 + \frac{4\nu_L}{R}\frac{dR}{dt} + \frac{2S}{\rho_L R} \tag{1}$$

As we can see, and following the equation (1) from left to right, the first term relates with the pressure in the system, the second one has to deal with the inertia of the system, the next term stands for the acoustic radiation of the system and its later response. Then the fifth and the sixth terms are composed by two terms, the last one determines the surface tension of the system and the viscosity term of the equation.

The forces that act on the viscous terms should be modified since, in this case, the medium is not a liquid such as in the Rayleigh-Plesset [74], in this thesis, the medium, as said before, is composed by gelatin. Therefore, the tensor denominated as Voigt term, has to be added to the model in order to take into account the properties of gels, such as in this case, and it properties like their viscoelastic ones. This equation, the Rayleigh-Plesset equation in addition with the Voigt term, (which consists on a spring term and a viscous damper) was taken from Hamaguchi's work [72]. One thing that has to be explained is the last term of the equation (2), where f is the frequency, A the amplitude of the wave, and the whole term remains for the wave that will insonate the bubble.

$$\rho\left(R\ddot{R}+\frac{3}{2}\dot{R}\right)=\left(P_0+\frac{2S}{R_0}\right)\left(\frac{R_0}{R}\right)^{3\kappa_{eff}}-\frac{2S}{R}+3\int_R^{\infty}\frac{\tau_\pi}{r}dr-(P_0+Acos(2\pi ft) \qquad (2)$$

Equation (2) should be linearized because A has to be small enough in order to provoke a linear oscillation behaviour in bubbles during the theoretical experiments (although this has to remains equal if the experiment is performed in real conditions), Once it is clear that linearization has to be done, parameters from the equations have to be mentioned, where μ_{eff} of (3) and κ_{eff} of (2) remains respectively for the viscosity and for the polytrophic index [72]. Then the parameter G stands for the gelatin's rigidity at environment conditions. Then the stress tensor will be evaluated according Voigt model. [83]

$$3\int_R^{\infty}\frac{\tau_{rr}}{r}dr = -\frac{4\mu_{eff}}{R}\dot{R}-\frac{4G}{3}\left[1-\left(\frac{R_0}{R}\right)^3\right] \qquad (3)$$

Because of the linearization, we linearized the equation as much we can obtain

$$m\frac{d^2x}{dt^2}+c\frac{dx}{dt}+kx=A\cos(2\pi ft), \qquad (4)$$

These kind of equation is leaved and solved as a generic solution in which some of the parameters have suffered a change of variables, which is the case of

$$m=\rho R_0^2, c=4\mu_{eff}, k=3\kappa_{eff}\left(P_0+\frac{2S}{R_0}\right)-\frac{2S}{R_0}+4G.$$

And as we are dealing in our thesis with a steady-state scenario the equation (4) can be even more simplified obtaining:

$$x(t) = X \cos(2\pi ft + \delta), \tag{5}$$

In which the phase shift δ is given by:

$$\delta = \arctan \frac{2\zeta(f / f_N)}{1-(f / f_N)^2},$$

With a damping ratio defined by ζ , and f_N which means the bubble resonating frequency at normal conditions.

$$f_N = \frac{1}{2\pi}\sqrt{\frac{k}{m}}, \quad \zeta = \frac{c}{2\sqrt{mk}}.$$

Also from (5) is needed to explain that X, is the amplitude of the signal, which according to the study of Hamaguchi & Ando [72] comprehends

$$X = \frac{A / k}{\sqrt{\left[1-(f / f_N)^2\right]^2 + 4\zeta^2 (f / f_N)^2}}, \tag{6}$$

Then, we can join them up together and obtain the resonating frequency of the bubbles.

$$f_N = \frac{1}{2\pi}\sqrt{\frac{3\kappa_{eff}P_0}{\rho R_0^2} + \frac{2S(3\kappa_{eff} - 1)}{\rho R_0^3} + \frac{4G}{\rho R_0^2}} \tag{7}$$

3.2. How does the frequency response vary regarding pressure changes?

Once the theoretical equations have been explained and all the terms of them understood, it is easy to notice that the radius of the bubble takes extremely importance in the equation, since all the terms depend on it. But this radius it is not only the only parameter that takes relevance in order to obtain the fundamental acoustic response. As we can appreciate, there are others such as the surface tension (S) or the polytrophic index (κ_{eff}), but the one we will focus on is on the ambient pressure, which takes place on the first term of the radical by the expression ofP_0. This term has extremely relevance because, as we explained in the past sections, according to Minnaert's work [30], as the pressure of the system changes, the acoustic response of the bubbles will change too. Another reason why this pressure term is so important is due to the rest of the parameters. If we take a look at them, we will see these ones are mainly constants that will be set according to the medium, but if the system is maintain the same, these parameters will no change, not as the radius nor pressure term, which are the only ones that are not constants regarding time. Then, considering Voigt model, Minnaert's [30] equations can be developed and simplified, but first, the resonance frequency deduced by Minnaert has to be consider as:

$$\omega_0 = \sqrt{\frac{3\gamma P_0}{\rho R_0^2}}, \tag{8}$$

In such a way, ω_0 gives the frequency of the system, and the adiabatic index γ. This last parameter, will be taken into account as 1.4, due to the system relies on a isentropic state. As this happens, we can consider the adiabatic index as polytrophic leaving the value as k = 1.4.

The pressure of the system has a direct relationship with the radius, thus, if the radius changes, is due to the pressure has changed too from the ideal Gas Law,. From this equation we can deduce the following:

$$P_0 R_0^3 = (P_0 + \Delta P) R_1^3,$$

Thus, in case the bubble is compressed with a ratio such as the increment of the pressure (ΔP) reaches the initial pressure (P_0), the fundamental frequency formula of Minnaert becomes incomplete and has to be implemented adding these second law. Once the Minnaert's formula for the resonating natural frequency is joined implemented by adding at it the Ideal Gas Law, a new equation is obtained which can be used for calculating pressures by relating fundamental frequencies:

$$\omega_1 = \sqrt{\frac{3\gamma P_0}{\rho R_0^2}} \left(1 + \frac{\Delta P}{P_0} \right) = \omega_0 \left(1 + \frac{\Delta P}{P_0} \right) \tag{9}$$

As we can see, it is possible to find the unknown pressure by knowing from advice the resonate frequency.

4. Thesis objectives

In order to introduce the experiment in which the thesis is based, first, the objectives shall be explained. The objectives that will be tried to achieve are the following:

- Developing a theoretical model in which any parameter of the Rayleigh-Plesset equation can be computed, this model comprehends:

    - Simulation of the system, which will be as realistic as possible.
    - Developing an algorithm in order to obtain the best results and to optimize accuracy.
    - Implementing the algorithm with advanced signal processing techniques for time, radius and pressure.

- Obtain theoretically the pressure by simulating the system at its whole
- To give graphic comparison of the different procedures and simulations performed, as well as the results obtained depending on the parameters introduced and see what is the best approach performed.
- To give a theoretical approach which would be used in the future to perform experimentally the same procedures.

5. Theoretical work and procedure

5.1. Rayleigh-Plesset equations

In order to prove that the bubbles behaves like linear oscillator, Rayleigh-Plesset equations will be linearized, since if the result linearized should have the shape of a linear oscillating behaving bubble. As it was explained before, and will be demonstrated later, this shape is characterized by the harmonic oscillator with damper. Our first aim is to obtain this goal. In the other hand, it is useful also to linearize the Rayleigh-Plesset equations due to get our final objective. We could calculate the pressure at which the bubble is located by determining the resonance frequency of the oscillator.

Therefore, the very first part of the experiment, consisted in by taking from scratch the Rayleigh-Plesset equations [38, 39, 74], both, with and without the Voigt model, for a later comparison of the results. From the original equation and the Figure 14, we tried to simplify it obtaining later the following:

$$R\ddot{R} + \frac{3}{2}\dot{R}^2 = -\frac{1}{\rho}(P_0 + P_a - P_B)$$

Where pressure P_0 is the pressure at the bubble's radiusR_0, $P_a$is the pressure at the origin of the system, which corresponds at the centre of the bubble if we put it at the origin of the system. Then, P_B will correspond at the pressure at the infinite, since we consider the bubble immersed in an infinite liquid or when Voigt model is used, gel-like fluid. R is the radius of the bubble, and $\ddot{R} and\ \dot{R}$are the second and first derivate of the radius according time respectively. As we use the continuity equation in a sphere and projecting the area of the bubble, we can deduce:

$$P_g = P_B + \tau_{rr} + \frac{2\pi}{\sigma}$$

In order to obtain an equation in which is possible to work with, we findP_B and then we first non-dimensionalize the equation knowing the oscillation amplitude is small enough in order to be neglected. Then the dimensionless equation will be:

$$\rho\left(aR_0^2\omega_0^2\ddot{a} + \frac{3}{2}R_0^2\omega_0^2\dot{a}\right) + P_0 + P_a - P_B = 0$$

Where a is the adimensionalized radius, and ω the non-dimensional time. As we can see, the radius has still their derivatives, but, since $R_0$is a constant, it remains the same. We also knew previously:

$$a = 1 + \ \varepsilon r(\tau)\ and\ \ \varepsilon \ll 1$$

Therefore, the correct expression for the non-dimensional equation is:

$$R_0^2\omega_0^2\varepsilon\ddot{r} + \rho(P_0 + P_a - P_B) = 0$$

And with Voigt:

$$R_0^2\omega_0^2\varepsilon\ddot{r} + \frac{4}{3}G\left[1 - \left(\frac{1}{1+\varepsilon r}\right)^3\right] - \rho(P_0 + P_a - P_B) = 0$$

This term introduced in the middle of the equation corresponds to the non-dimensionalization of the Voigt model. Thus, once this is obtained, both equations are linearized following a Taylor expansion of second degree, since a bigger degree yielded same results, therefore, we searched also to be as efficient as possible.

Once the equations were linearized, as previously anticipated, both results could be resumed on the equation of a harmonic oscillator with damper, and by the way they have this shape:

$$\ddot{R} + \beta\dot{R} + \Omega_0^2 R = -\cos(\omega t)$$

Where $\beta$and $\Omega_0^2$are constants that follow the derivatives of the radius. For obtaining this result, was necessary to dimensionalize the equations once the equations were linearized. Therefore, this showed us we were on the right path, since the results of the theoretical solutions showed us, the bubble, when resonating, behaves like a harmonic oscillator with damper, which is correct as Aldham [34] achieved on his theoretical results.

Then, we integrate this solution over periods of$\tau = 0$; $\tau = 2\pi$ $\tau = 10\pi$ and $\tau = 50\pi$ obtaining results that will be commented.

5.2. Simulation

As before was said, this thesis relies on the theoretical work behind the experimental part, in other words, this thesis is a theoretical simulation of the behaviour of the bubbles when insonated, with the final aim of obtaining all relevant parameters and obtaining a relationship between the pressure and the natural response of the bubble once they are insonated by the pulse, showing and discussing of the results.

Therefore, this theoretical approximation has to be explained, and what would it be what we are simulating on this thesis.

First of all, could be resumed in four main parts, the first one describes the medium used and its preparing. The second one lies on the bubble generation and the fourth one the insonationg of the medium.

These three steps are the previous preparation of the experiment which are needed in order to obtain the first results. This first results will be later processed computationally by a program such as Matlab as in this case in order to obtain the real measures and the signal processing needed. This procedures are also simulated in Matlab for this thesis as said before, but in other experiments that could be carried on knowing in advice this thesis, and if they want to make it experimental, instead of programming this procedures would have to do them manually.

When preparing the medium, is useful to know what are the purposes, and since we want to achieve similar results as the human body real tissues, our medium will be composed by a gel-like medium that will have more or less the same properties as the medium we want to test. As we cannot obviously use human gelatin (only if the procedures are performed experimentally), in this case we perform the experiment first with porcine gelatin from the skin, since we had the values previously in the lab, and in other researches such as the work done by J. M. Solano-Altamirano[73]. It is a fact that since we are theoretically resolving the problem exposed, we could simulate the soft human tissue, and will later be shown the results. This results were not much different than the ones obtained with porcine gelatin, since our evolutionary differences in this aspect, are not significant.

Once the medium is prepared, the next step is the bubble formation. In case is done theoretically, it will be much easier, since there is only to program the bubble's behavior by the Rayleigh-Plesset equations. This procedure is characterized by the programming of what in the previous paragraph was done manually. It is needed to build a program which will adimensionalize the equations (in this case only the Voigt term added equation, since we are dealing with gel-like mediums, thus, this term is must needed) and once the equation is adimensionalized, solving the differential equation and integrating it throughout its corresponding time lapse or the periods in when we want to study and plot them.

In the other hand, if we want to make this research theoretically, the procedure of creating the bubbles is much more difficult and wearisome than the programmed one. First, if the gel-like is put on the desired recipient, which has to be thick enough to support the compression of the medium and also the pulses emitted by the oscilloscope, as in this case the chirps. Once this is performed, a laser pulse has to be focused on the phantom. This laser pulse has to be generated by using high-power methods, in which the laser beam reaches the 100 mJ of energy and has to be emitting during almost 1 second.

The point where the laser beam is focused starts to warm quickly reaching a point where the gel-like medium burst in a small point, yielding little cavities that will oscillate. This process is called cavitation, in which bubbles are formed by the warming of the fluid, causing a difference of temperature and making small areas where this temperature rises to burst. Once this cavity is formed, it will oscillate by compressing and expanding until reaches an equilibrium. At that point, bubbles will be formed. We can see in Figure 15 how these bubbles are created due to cavitation.

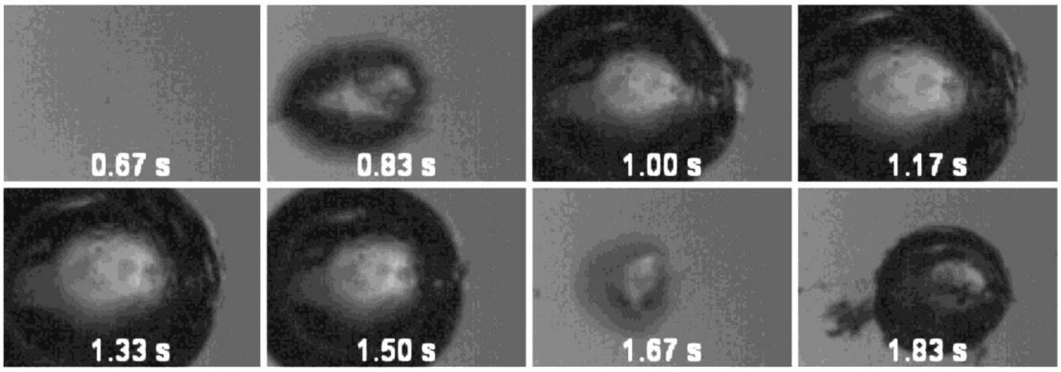

Figure 15. Process of bubble cavitation by temperature using laser technique [45]

As we can appreciate in the Figure 15 once the laser beam is focused on an area, this area burst and this cavity oscillates until at the 1.83 seconds finds its equilibrium becoming a bubble.

Another way of cavitation which does not need a temperature increase, is when air is injected on the medium. This process is quite different than the previous one, and consists in injecting air with a really small radius needle, with about 0.5 mm. This technique also creates the bubbles, but is less scientific than the previous one, since the bubbles created are different ones from another, because of the human errors. Also, the bubbles created are not spherical, while in the cavitation by temperature, when reaching the equilibrium, the bubbles become spherical. The conditions for performing experiments with them are not the ideal (when injected) while when using the laser beam technique fulfil them.

Then, the third part is the insonation of the system. As told before, the chosen option to the pulse was a chirp, because of the properties explained before. Usually, a wave generator is in charge of the acoustic pulse emitting. As in this case, when chirps are used theoretically and experimentally, both, have to be programmed. In the case of this thesis, a chirp was programmed to be emitted by the own Matlab. This program was created using as inputs, the duration time of the pulse and the frequency ate which the bubble resonates as we wished. Then a sinusoidal wave with damping was created. We thought a sinusoidal wave was the best option since starts at zero in the coordinate axis. As the chirp has a sinusoidal shape, it will cover high frequencies and it will be at first more noticeable until time passes and starts to oscillate at a lower frequencies, covering the range we desire. This time that the chirp lapses is a short period of time, about 0.1 seconds, but this will depend regarding how this pulse is programmed. In the case of this thesis, is programmed to have a length of 0.15 seconds.

Then, because of the chirp, (the chirp which appears in Figure 13 was used for the experiment) the Figure 16 first oscillates at higher frequencies, as we can see when the radius decreases and grows more at the beginning, and then oscillations start to shrink as time passes until the bubble reaches the equilibrium. This pulse wave is very important for the thesis since without it, no oscillation would be possible, and neither to obtain any result.

In the other way, if the procedures are performed experimentally, all oscillations should be recorded in order to results for being obtained. This recordings later processed through a computer using programming tools such as Matlab or Python for a signal processing in order to pressure to be calculated.

5.3. Computer algorithm

Once the numerical calculations were performed, and the results were successfully, since as explained before, these was comprehend in what we believe what was going to be, and as expected, an harmonic oscillator was described. Therefore, we performed an algorithm in which we desired to obtain the same results but, in this case, computerized results. Thus, we built the algorithm in Matlab, because it fulfil our aims for computing differential equations and perform some other calculations. We also decided to write the code in Matlab because this platform is mainly used for calculus solving and the facilities provided by the program are huge enough to be decided. It was also chosen because of it is incredibly feasibility for dealing with signal and imaging processing in which we were really interested in.

Therefore, by using the command Ode45, solving differential equations was successfully possible. We developed a program also, in order to solve at once the differential equation in a more accurate way. This program received all the necessary inputs, which were the parameters of the system, such as the density and the viscosity of the medium, measured in [kg/m^3] and [Pa s] respectively, that because of the use of the Voigt model, a gel-like medium was able to be used. Its shear modulus [Pa], the initial radius of the bubble measured in [m], that because we were dealing with microbubbles, its size rounded the 100 μm. There were other parameters the program took into account, such as the surface tension of the bubble [N/m], the ambient pressure measured in [Pa], the polytrophic index which had no units, and since we saw before, this parameter can be considered as k = 1.4, since the theoretical oscillations we will be deal with are isentropic, therefore, adjusting the adiabatic index as a polytrophic index with value 1.4, will make everything much easier, which also was considered by Prosperetti on its work [44]. Other parameters that also were defined as inputs were the pressure amplitude defined in [Pa] and the sound angular frequency with Hertz’s as units.

The program, returns two vectors called U and dU, which will contain the radius variation and the time respectively. Therefore, we are obtaining both variables, solved, respect the time.

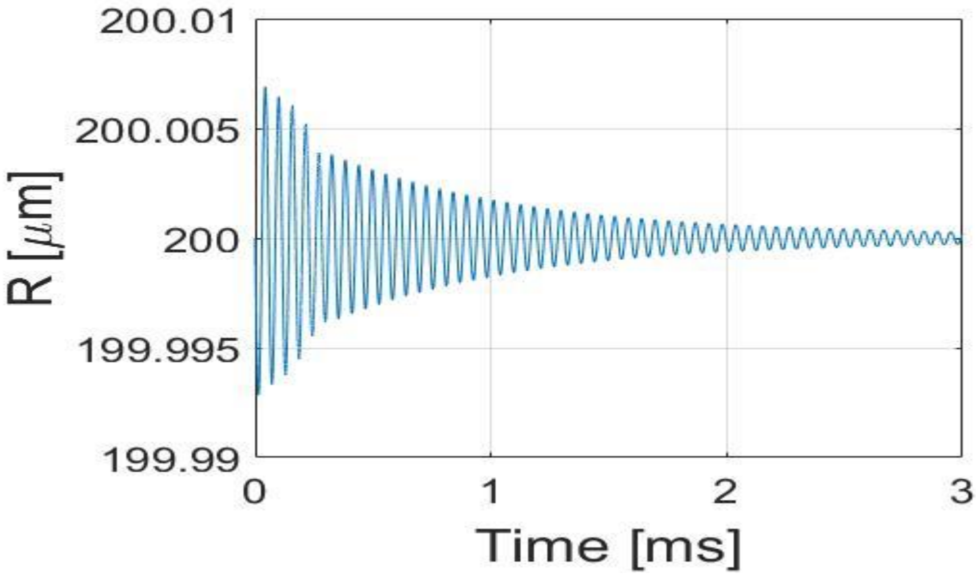

Figure 16: Plotting of the radius of the bubble against time when insonated by the chirp shown in Figure 13.

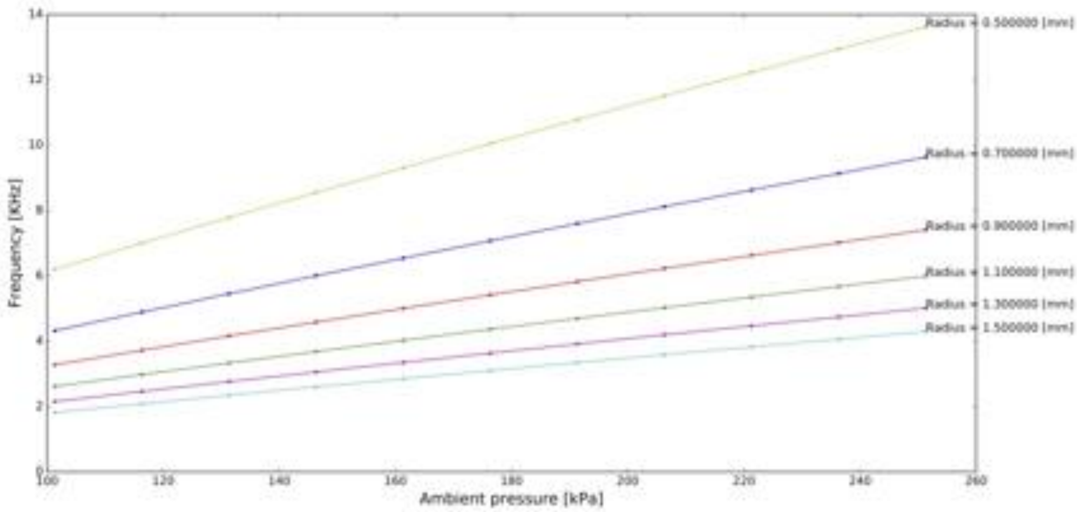

Figure 17: Plot that shows how the frequency varies according the pressure changes. [40]

But, one variable which is as important as the rest of them is the chirp. This chirp, as explained before, is an acoustic pulse which comprehends a huge range of frequencies which can be perfectly appreciated in Figure 13 This chirp is the best pulse option for insonating bubbles, since provokes the desired response we want. As previously was explained, as the chirp has a period during time of 0.15 seconds in which high frequencies (in this chirp case) are emitted and insonate the programmed bubbles in the desired medium. As we can see from the Figure 14**,** the bubbles starts to oscillate once the chirp insonates it, but as time passes through, the radius variations start to shrink leading the bubble to become in equilibrium phase, which was the previous phase before the chirp was emitted. Going a bit back, the timing vector response, gave us a vector of time values, but these values were no the right ones to operate with, since these values are not equispaced in time. As we later will need to compute the fast Fourier transform in order to evaluate the frequencies range. The main problem we dealt at this point was the needed of linearize the vector. The vector has to be interpolated in order to fulfil this condition. Therefore, for determining the frequency at which bubbles resonates, an analysis of the spectrum is needed. For this reason, in order to obtain this analysis, the Fast Fourier Transform was applied on the oscillation of the bubble's radius obtained in the vector in which the solution of the differential equation is given. But as explained before, the time has to be equispaced in order to perform this analysis. The way we performed this was first calculating the number of samples at which the interpolation should be done. This number of samples had to be equal at the dimensions of both vectors that were given as outputs. Once this samples are obtained, the way we equispaced the dimensions was by using the *interp1* function in Matlab. This function returns one dimension interpolated values at certain points which were introduced on the same. Therefore, the number of numbers at which the values are interpolated graphically is a modifiable variable.

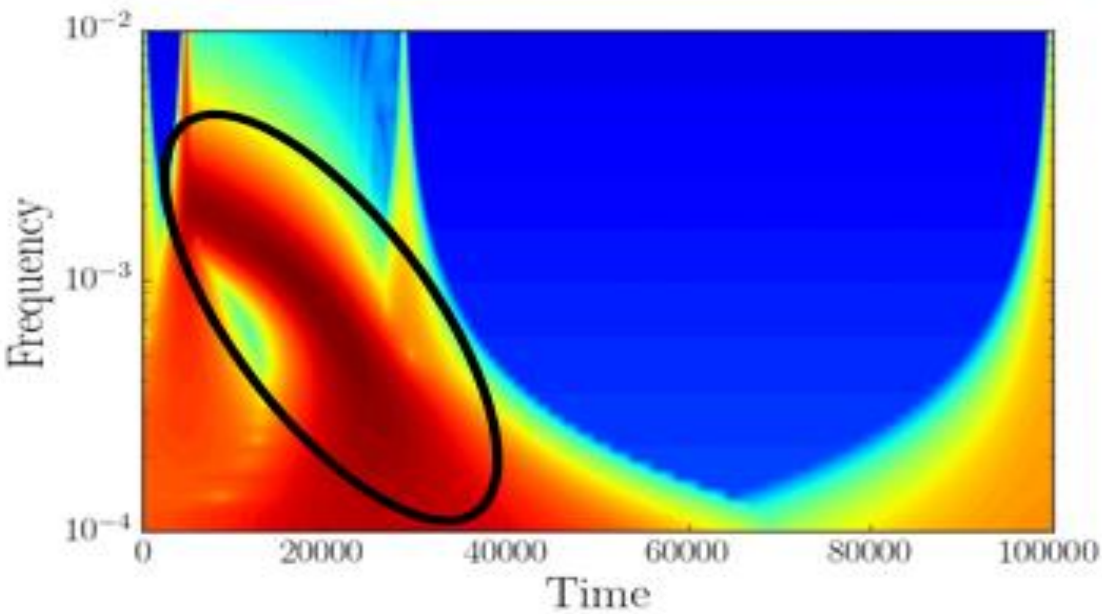

Figure 18: Plotting of the frequencies of the signal against time. The black oval represents the area where the central frequency of the signal is focused on. [45]

Once we obtained the equispaced time, we could perform the frequency spectrum analysis as desired. For it, we decided the best approach was to perform the Welch's method, an estimation of the power of the signal introduced at a range of frequencies. This is an approach to the spectral density estimation, which estimates as it is said the power spectrum, in other words makes a probability function of the distribution of the power with the help of the range of frequencies of the signal according to Fourier analysis in terms of wave analysis. This procedure yields the frequencies spectrum versus its time, which is based on wavelets analysis according to Haar [75] as it is represented on Figure 18. This analysis is quite different than the Fourier spectrum analysis in terms of duration, the one used is not prolonged infinitely on time and is not based on a series of sines or cosines, while in the Fourier spectrum analysis, the spectrum is prolonged infinitely on time and is based on sine or cosines series. This sines or cosines series try to approach an estimation of the original signal. This Power Density Spectrum, was performed by the Welch's model, using the *pwelch* Matlab function. The window used was varied and then the results will be commented and exposed in the next section. This function gave us back two vectors, one containing the distribution of power per unit frequency in a vector array, and the other variable returned was a vector in which frequencies in Hz at which the Power Spatial Density is estimated. Then the window size input was always a potency of 2 yielding to better results. Also the number of overlaps in which the function operates was always defined by the half value of the windowing introduced. Depending on the window, the results are different, since the power spectrum is evaluated in other positions, something which will be noticeable in the next section when results are commented and explained. Then, the spectra was graphically explained by plotting the distribution of power per frequency unit in dB against the sample of frequencies estimated evaluated over a period Figure 19.

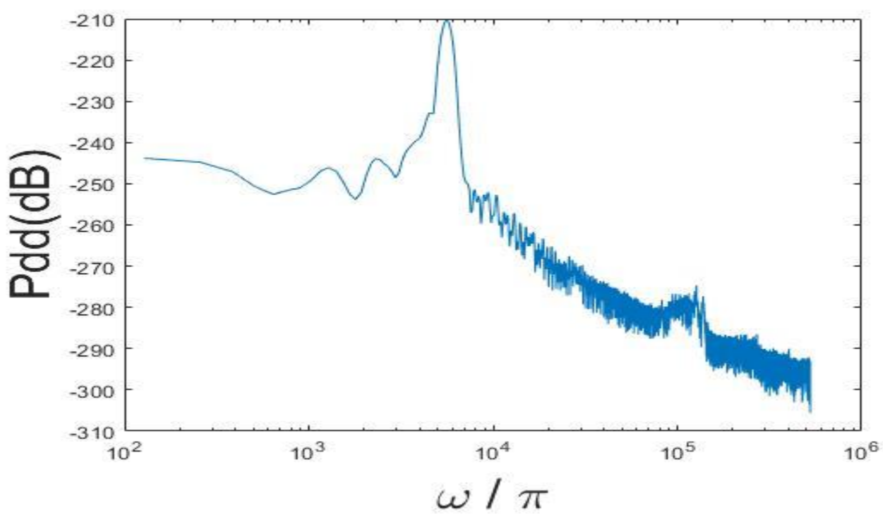

Figure 19: Plotting of the Power Spatial Density against the sample of frequencies obtained using Welch's method.

Once the algorithm is performed for several windows, the peak exhibit in the graphic, is measured for each window and then compared with the other peaks of the different windows in order to make a better approach and understand better it. Also, the wideness of the peaks is compared for every window introduced. This wideness is measured according to the Shannon-Hartley theorem [73]. This theorem is an application of the communication theorem for noise channels that presents Gaussian noises. This theorem makes easier the computation of the wideness since stablishes the power distribution as a channel. Therefore, the results were obtained and later exposed on the next section where they will be compared. This results have a lot of relevance since depending on the window, the approach will be better or worse, and as we want this thesis to be continued experimentally, the best algorithm has to be implemented in order to obtain the best results, always trying to be as more realistic as possible. This peak measurement is calculated and tried to reach the best result in order to determine the pressure of the system. Which will be equal to the root of the amplitude of the peak, according to Minnaert [30]

Also, several mediums were simulated and results obtained. The medium used by Prosperetti and Hamaguchi [44, 72] was firstly used and then compared with variations on the system to see and understand the behaviour of it and the changes on the parameters. After this, a simulation of a gel-like medium (porcine gelatin of the skin) was done and also compared with the human gelatin. This last step was done in order to see and understand two things, the first thing is to understand and make an approach of the bubble’s behaviour when in human soft tissues, which is the main application that wants to be done of the problem. The second reason is to demonstrate the low differences of the porcine and human tissue according to evolution.

6. Results and Discussion

As explained before, the simulated procedure was done in order to calculate the pressure of the medium surrounding the microbubbles when these are insonated by an acoustic pulse, in this case the one used was the chirp because of its huge range of frequencies. This chirp was programmed to comprehend a range of frequencies from 0 to 1 kHz. Therefore, according to the variation of radius. Then, the resonating frequency of the bubbles is seen in the results given, and it can be computed as variations of the radius according time. This frequency can be also calculated from the equations described in the Section 3: Theoretical Background. This equations should have included on it the Voigt term, since all the mediums in which we were dealing with, were gel-like mediums. Also as was mentioned in that section, we wanted previously to check whether if the microbubbles satisfy the ideal gas law before they were theoretically insonated by our chirp. This simulation was performed by Matlab and it satisfied the theoretical calculations in the Figure 20 below.

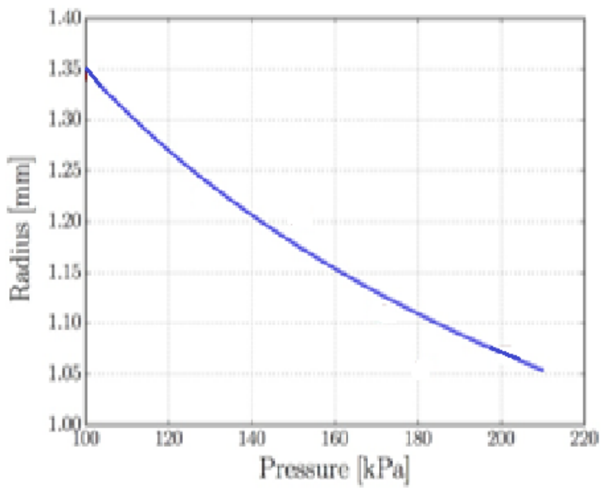

Figure 20. Plot of the radius evolution of the bubbles against the pressure change before bubbles were insonated, satisfying the Ideal Gas Law.

As explained before, at first, we performed the simulation scenario of bubbles being insonated by our programmed chirp. These simulation took into account Voigt terms since the medium in which we were dealing with was a gel-like medium, which firstly was programmed according the parameters used by Hamaguchi & Ando [72] with polytrophic index of $\kappa = 1.4$ which was obtained from the approaches done by Prosperetti [44] which corresponds to Figure 21.

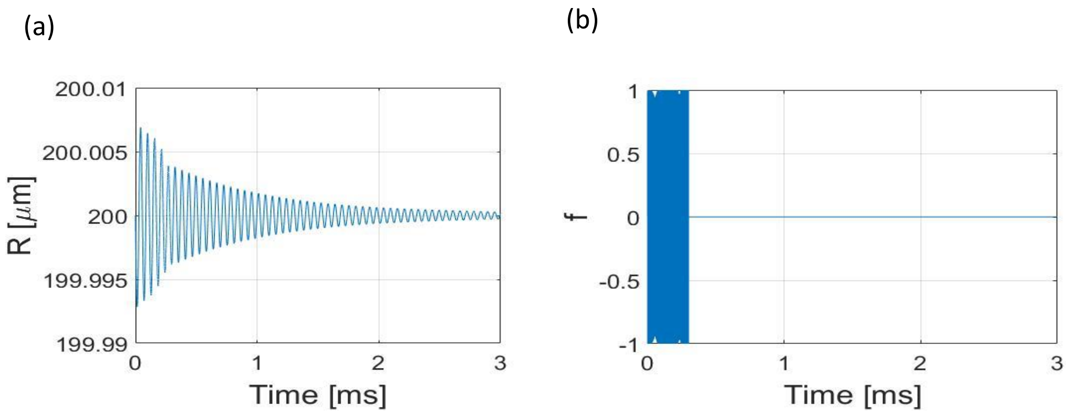

Figure 21: a) Plot of the radius variation of the coated bubbles against the time evolution of 3 ms when insonated by a programmed chirp b) that covers a range of frequencies from 0 Hz to 1 kHz. All these parameters are comprehend by the approach of Hamaguchi [72] and using the polytrophic index used by Prosperetti [44] of $\kappa = 1.4$

Thus, we also wanted to see how the radius of these bubbles varies according to other parameters in order to see and understand better the behaviour of the parameters such as the viscosity or the surface tension of the own coated bubbles. Therefore we simulated again other two scenarios in which all the parameters were varied. These behaviour will be appreciated and explained in the section of conclusions. It is very interesting to see how this microbubbles vary their radius differently using the same programmed chirp depending on the viscosity of the system for instance. As we can see on Figure 22 a), when the viscosity becomes lower, the oscillations start to attenuate previously on time with same acoustic wave insonating.

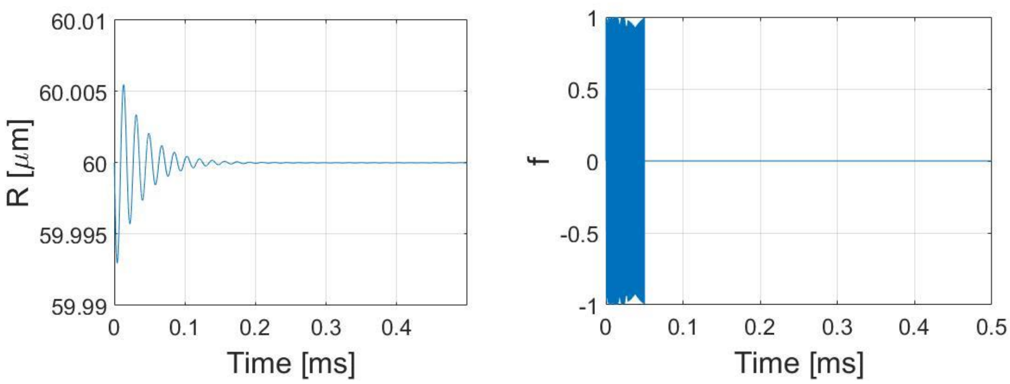

Figure 22: a) Plot of the radius variation of the coated bubbles against the time evolution of 0.5 ms when insonated by a programmed chirp b) that covers a range of frequencies from 0 Hz to 1 kHz. The parameters introduced on the system are different than the previous figure, All these parameters are $\rho = 953 \left[\frac{\mathrm{kg}}{\mathrm{m3}}\right]$; $\mu = 46.7 * 10\mathrm{e}-3$ [Pa $*$ s]; $\mathrm{G} = 3.2 * 10\mathrm{e}3$ [Pa]; $R_0 = 60 * 10\mathrm{e}-6$ [m]; $\mathrm{S} = 0.07$ [N/m]; $P_0 = 1$ atm; $\mathrm{A} = 1 * 10\mathrm{e}3$ [Pa]; $\omega = 28 * 10\mathrm{e}3$ [Hz]; $\kappa = 1$

(a) (b)

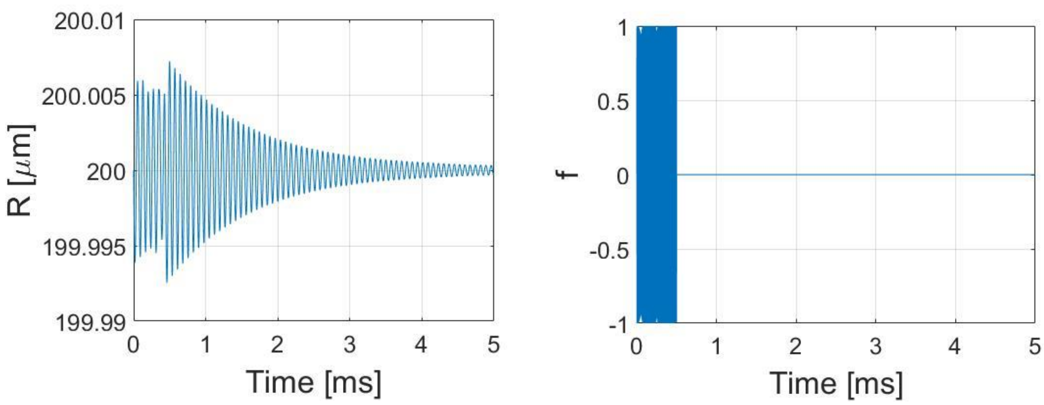

Figure 23: a) Plot of the radius variation of the coated bubbles against the time evolution of 5 ms when insonated by a programmed chirp b) that covers a range of frequencies from 0 Hz to 1 kHz. The parameters introduced on the system are different than the previous figure, All these parameters are

$\rho = 1050 \left[\frac{\mathrm{kg}}{\mathrm{m3}}\right]; \mu = 25.1 * 10\mathrm{e} - 3\ [\mathrm{Pa} * \mathrm{s}];\ \mathrm{G} = 5.1 * 10\mathrm{e}3\ [\mathrm{Pa}];\ R_0 = 200 * 10\mathrm{e} - 6\ [\mathrm{m}];\ \mathrm{S} = 0.02\ [\mathrm{N/m}];\ P_0 = 1\ \mathrm{atm};\ \mathrm{A} = 1 * 10\mathrm{e}3\ [\mathrm{Pa}];\ \omega = 28 * 10\mathrm{e}3\ [\mathrm{Hz}];\ \kappa = 1.6$

As it can be appreciated in Figure 23 a) since the radius is bigger than before, it needs more time to attenuate, in other words, the system needs more time in order to become again in equilibrium state. This Figures 21, 22 and 23, are useful in order to understand how bubbles behave when acoustic waves are propagated throughout the medium. As we can see, when the radius is low, the system lasts less time in order to go again to its original state. Also when the density of the system $\mathbf{\mu}$ is lower, these attenuations start before. This behavior was previously explained by scientist such as Minnaert [30] or Aldham [34] between others, thus this results are the ones expected for these bubbles since the control of the chirp frequencies was successful, since it was totally programmed by Matlab and all these procedures, as said before are simulations.

In order to understand better the working of these oscillations provoked by the programmed chirp, a Fourier analysis was performed and the results shown in the Figure 24. This Fourier analysis was performed by computing the Power Spectral Density as said before, following Welch's model, yielding to a resulting plots in which the frequencies spectrum are plotted against time according to Haar researches in wavelets.[75] In this power density spectra, the window size was varied in order to see whether the best approach is. All this window sizes are potencies of 2. Depending on the window size, the shape of the spectra will be different than another with a different window, since the spectrum is evaluated in other positions than the rest. It also has to be taken into account, that all these graphics have no units since the variables plotted are dimensionless. Also, from the spectra of Figure 24, can be easily appreciated that the peak is always found in the high frequencies, which makes sense, since the maximum oscillation of the bubbles, occur at low the starting of the insonation of the pulse to the bubbles, thus is consistent.

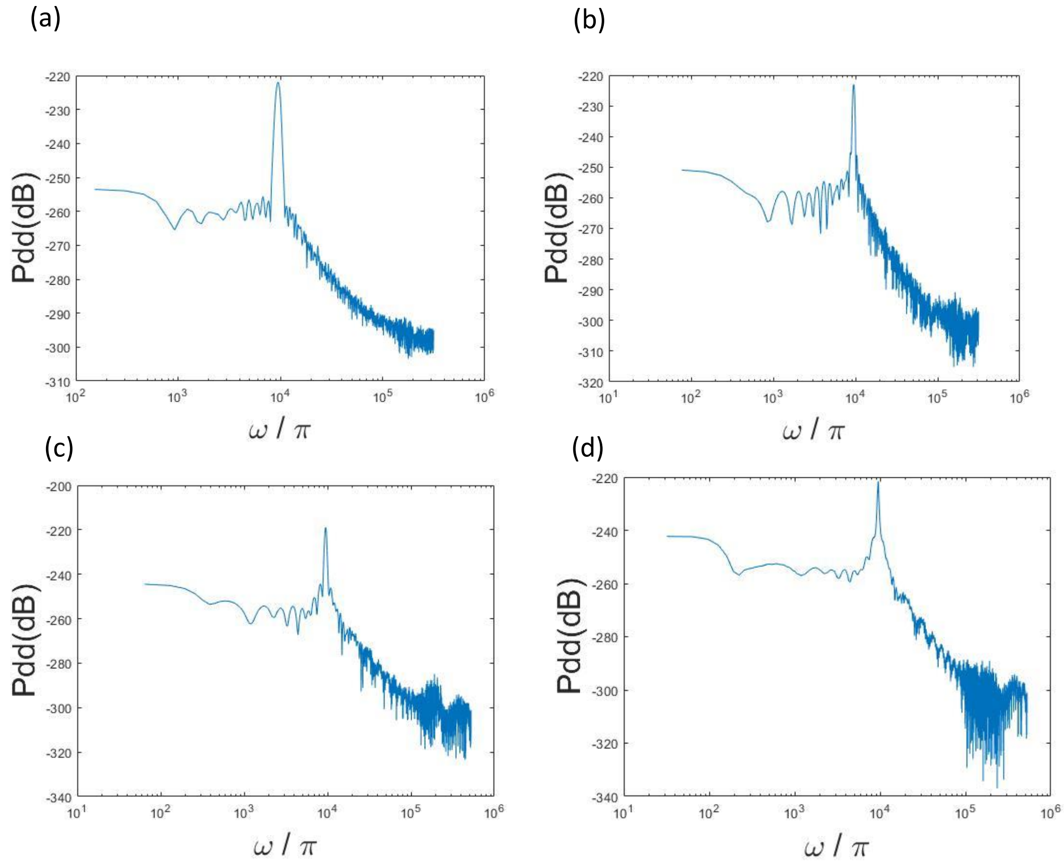

Figure 24: Several spectra which represent the Power Spectral Density following Welch's method by plotting the distribution of power per frequency unit in dB against the sample of frequencies estimated evaluated over a period. These results are taken with windows a) 1024, b) 2048 c) 4096 and d) 8192

As it is possible to see in Figure 24, as the window size is bigger, the more values it will take. This results have a lot of relevance since depending on the window, the approach will be better or worse, and as we want this thesis to be continued experimentally, the best algorithm has to be implemented in order to obtain the best results, always trying to be as more realistic as possible. Thus, it will be more accurate to represent the shape with windows with values c) and d). This will make much easier to perform and calculate the pressure measurement by using the Shannon-Hartley theorem [73] by taking into account the wideness of the main peak. As said before, this theorem is an application of the communication theorem for noise channels that presents Gaussian noises. This theorem makes easier the computation of the wideness since stablishes the power distribution as a channel. This peak measurement is calculated and tried to reach the best result in order to determine the pressure of the system. Which will be equal to the root of the amplitude of the peak, according to Minnaert [30].For this reasons, we could say the more relevant results are the ones in which the window size is higher, c) and d).

In order to be as realistic as possible, and since the aim of the thesis is to develop a solution for calculating the pressure of some areas where are difficult to measure in medical applications such as the beginning of the aorta for instance, all these calculations will be done with two mediums. In the first one, the scenario which is simulated, is the muscles of the pork, this medium is simulated by introducing the data of porcine muscle gelatin in which the bubbles will be theoretically found, Figure 25. As the window sizes of both 4096 and 8192 are useful to apply the Shannon-Hartley theorem [73], both Figures 25 c) and d) will indicate respectively the spectra of the Power Density of the bubble's oscillations within the porcine muscle gelatin.

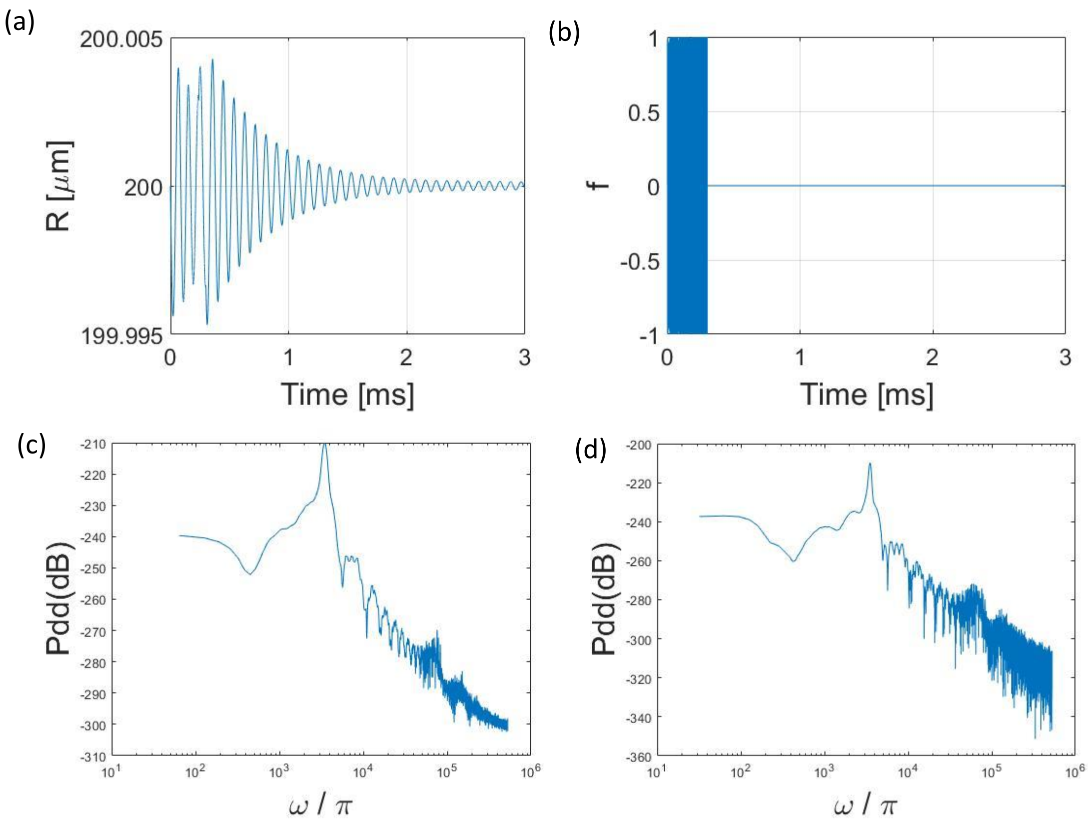

Figure 25: a) Plot of the radius variation of the coated bubbles against the time evolution of 3 ms when insonated by a programmed chirp b) that covers a range of frequencies from 0 Hz to 1 kHz. All these parameters are comprehend by the approach of Solano-Altamirano [73] for simulating a porcine muscle gelatin medium also with a polytrophic index of $\kappa = 1.4$ [44]. Spectra which represent the Power Spectral Density of a) following Welch's method by plotting the distribution of power per frequency unit in dB against the sample of frequencies estimated evaluated over a period. These results are taken with windows c) 4096 and d) 8192

On the other hand, the results obtained by simulating the medium of human muscle gelatin are the ones on Figure 26.

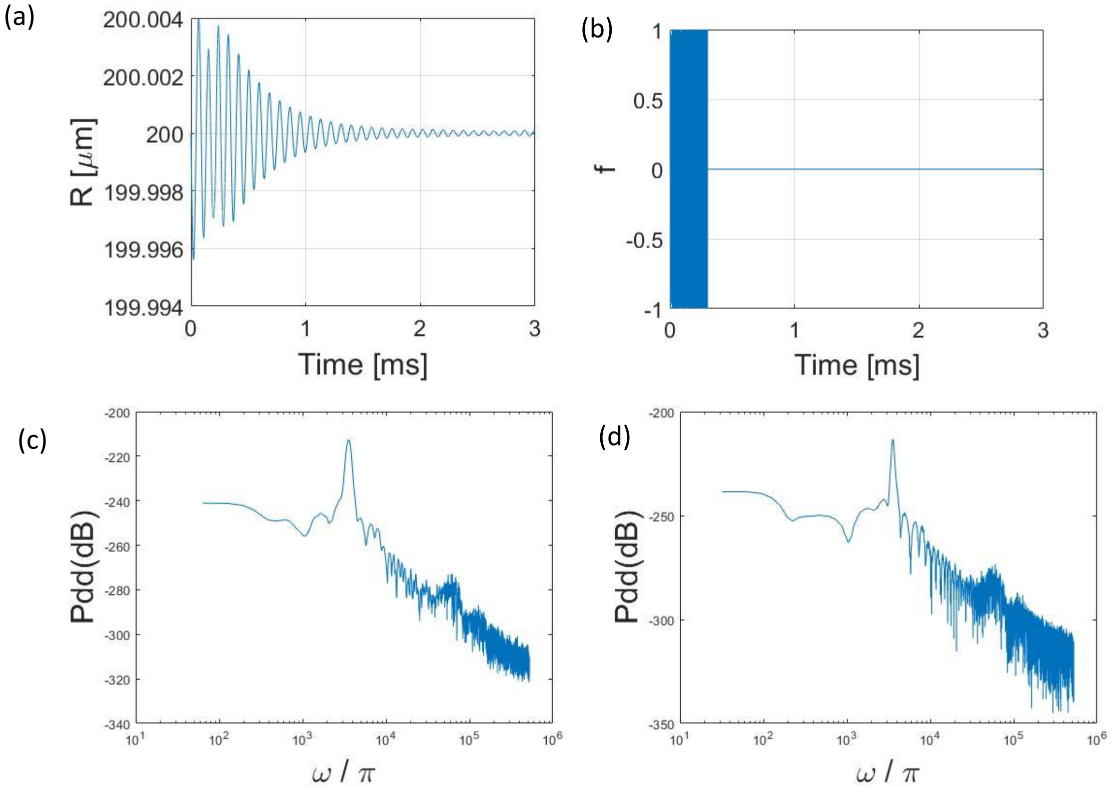

Figure 26: a) Plot of the radius variation of the coated bubbles against the time evolution of 3 ms when insonated by a programmed chirp b) that covers a range of frequencies from 0 Hz to 1 kHz. All these parameters are comprehend by the approach of Solano-Altamirano [73] for simulating a human muscle gelatin medium also with a polytrophic index of $\kappa = 1.4$ [44]. Spectra which represent the Power Spectral Density of a) following Welch's method by plotting the distribution of power per frequency unit in dB against the sample of frequencies estimated evaluated over a period. These results are taken with windows c) 4096 and d) 8192.

Therefore, as it can be appreciated, there are not mainly differences between Figures 25 and 26, since pigs and humans are close related in evolutionary terms. Thus, the pressure at which the bubbles are exerted at both scenarios, the one with porcine muscle gelatin and the one with human muscle gelatin, were calculated following the Shannon-Hartley theorem [73], and as it was said in the previous section, when noise channels present Gaussian noise, such as this case, the theorem results an application for communications. Due to the theorem is able to compute easily the wideness of the main peak resulted from the spectra by stablishing the Power Spectral Density as a channel. This peak measurement is calculated and tried to reach the best result in order to determine the pressure of the system.

According to the theorem, the pressure will be equal to the wideness of the peak, as explained before. The way it is said by the theorem in order to be the wide of the peak calculated is to divide the sample frequency over the window used for the Power Density Spectrum calculation. Obtaining the following results for both cases, demonstrating the low evolutionary differences between the two species and in such a way, this procedure at a whole is efficient for calculating the pressure at which bubbles are exerted within the system.

The pressure at the parameters introduced said in Figures 25 and 26 with the chirp programmed with a range of 0 Hz to 1 kHz that lasts 3 ms exerted from the medium to the bubbles is of 0.5 kPa in the Human simulation compared with the 0.48 kPa in the porcine medium simulation as it can be seen in the Table 3 below.

Organism	Window 4096 pressure (kPa)	Window 8192 pressure (kPa)	Average pressure (kPa)
Pig	0.46	0.5	0.48
Human being	0.47	0.53	0.5

Table 3. Pressure measurements results obtained by using the Shannon-Heartley theorem in porcine and human simulations.

The accuracy of this procedure relies on the procedure performed. As in this case we have used the biggest windows sizes for the Fourier analysis, the accuracy for the measurement is the highest we could achieve. On the other hand, our pressure calculation was carried out theoretically, thus, the results obtained will not have human errors, which determines more accuracy whether the experimentally pressure calculation using US, as the ambient pressure varies constantly, human errors occur and the instruments used for measuring such as the high speed camera have relative errors. This premise does not say our experiment is empty of errors, since the pressure was calculated by performing an average between the thickness of the peaks of both, 4096 and 8192 window sizes.

7. Socioeconomic contest

Pulmonary and cardiovascular diseases are one of the deadliest diseases, indeed cardiovascular diseases are the first disease dead cause in women. Therefore, their detection for a correct anticipation and treatment is crucial in order to save lives. As before was explained, some areas of the body have very difficult access because these areas are located in the very intern part of the body and limit with many organs, or due to the high risk of provoking any damage to function needed organs. Therefore, the measure of the pressure in these difficult access body locations has to deal with diseases such as the pulmonary arterial hypertension as it was explained in section 2. In case of this disease, can also lead to blood intoxication by the increase and amount of carbon dioxide in blood. This carbon dioxide bounds the hemoglobin present in the red blood cells and intoxicating them and provoking hypercapnia. By the moment, the only effective way to measure with a certain accuracy is by measure physically in the heart, therefore using an invasive way.

It is also useful to say, Ultrasound Contrast Agents have been proved as a safety in all these years of use in medicine [76], indeed, they have tested as a very safety compounds for non-invasive procedures, although introducing new chemical compositions and formulations regarding this application could be difficult since the authorities are strict for the approval to new techniques in terms of health.

This thesis has proved the efficiency of UCA's (taking into account their micro bubble's behavior) for a non-invasive and harmfulness way to measure pressures in difficult access body locations with a high accurate results. As commented in the end of the previous section, this was achieved because of the theoretical work it is itself this thesis, but it will be needed an experimental procedure in order to gather the effectiveness and the real accuracy of the pressure measurement of the procedure, since it will be experimental and not theoretical as this one.

For the performance of the procedure of this thesis, Matlab software package was used. This software package was offered by the university platform, and the main reason for using it was the facility of the language used and its feasibilities. In order to obtain the linearization of the equations, the solution for the Rayleigh-Plesset equation and the Fourier analysis it is useful to use a mathematical tool such as Matlab, and we thought all algorithms which were built will be used in the future once the empiric experiment will be performed.

In order to the experimental part of this thesis become reality, some other tools will be needed such as a high-speed camera for acquiring as many frames as possible since the oscillations occur at the order of microseconds. The procedure of how this could be carried out is also explained in section 5.2. Simulation.

Focusing on the economic environment of the thesis, the duration time of it was about 3 months and the costs would be the followings:

HUMAN RESOURCES COST	Cost (€/hour)	Number of hours	Total Cost (€)
Student	10.5	200	2.100

The salary is taken by the minimum wage perceived by working on the Universidad Carlos III de Madrid as a postgraduate research project.

Also for the materials employed:

Material	Cost (€)
Computer	1000

Total Cost (€)	3.100

As we can see, this project would be very suitable, since we achieve the results we gathered and the costs would be minimum.

8. Conclusions

From the objectives proposed in section 4, as it can be appreciated, we have fulfil all of them:

- Developing a theoretical model in which any parameter of the Rayleigh-Plesset equation can be computed.
- Obtain theoretically the pressure by simulating the system at its whole
- To give graphic comparison of the different procedures and simulations performed, as well as the results obtained depending on the parameters introduced and see what is the best approach performed.
- To give a theoretical approach which would be used in the future to perform experimentally the same procedures.

This thesis, has proved that theoretically, the pressure at which the bubble is located can be measured when the bubble is insonated by an acoustic pulse. The estimation of the pressure has been as realistic as possible taking into account several parameters that will affect the response of the bubble. Due to these reasons, we can say the accuracy of the pressure measurement has been good enough since we tried to use all parameters that interfere in real life for obtaining the best result.

Nevertheless, this experiment has to be tested empirically for being realistic and deciding if its accurate enough for being used in the near future as a non-invasive and harmfulness technique for measuring the pressure at difficult access body locations.

Due to this reason, as a future work, all what is said in section 5.2. Simulation, can be performed and even improved for getting the best experimental approach for calculating the pressure using US where bubbles are located and in such a way, constituting a fully non-invasive medical application.

9. References

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